(2)

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : G01N 1/31 — A2

(11) International Publication Number: WO 00/66995

(43) International Publication Date: 9 November 2000 (09.11.00)

(21) International Application Number: PCT/US00/11371

(22) International Filing Date: 29 April 2000 (29.04.00)

(30) Priority Data:

60/131,660	29 April 1999 (29.04.99)	US
60/155,299	21 September 1999 (21.09.99)	US

(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

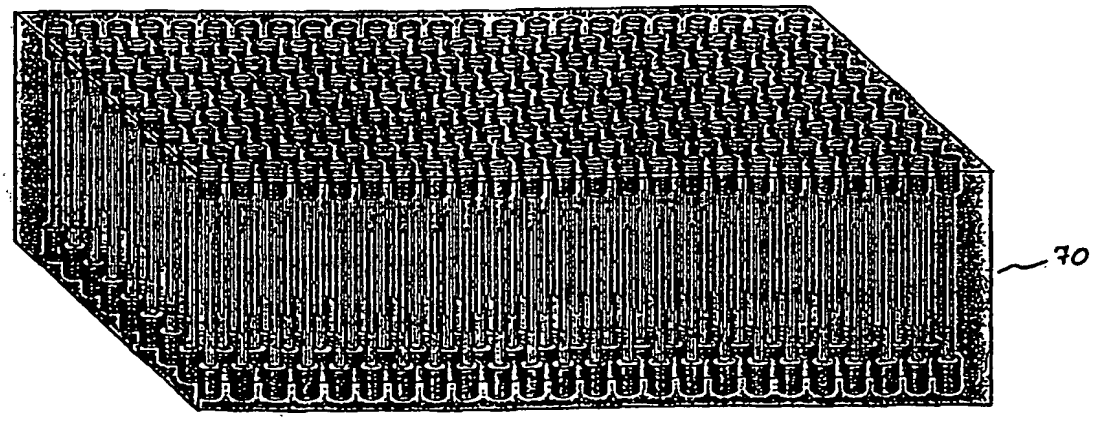
(71) Applicant: GENOME THERAPEUTICS CORPORATION [US/US]; 100 Beaver Street, Waltham, MA 02453 (US).

(72) Inventors: CAHILL, Patrick; 42 Elliot Street, Natick, MA 02160 (US). SMITH, Douglas; 2 Mayflower Lane, Gloucester, MA 01930 (US). THOMANN, Ulrich; 110 Shade Street, Lexington, MA 02421 (US). ENGELSTEIN, Marcy; 48 Fuller Brook Avenue, Needham, MA 02192 (US).

(74) Agents: LAURO, Peter, C. et al.; Lahive & Cockfield, LLP, 28 State Street, Boston, MA 02109 (US).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: DEVICE FOR RAPID DNA SAMPLE PROCESSING WITH INTEGRATED LIQUID HANDLING, THERMOCYCLING, AND PURIFICATION



(57) Abstract

The present invention provides an automated device to facilitate rapid processing of sub-microliter samples using chemical or enzymatic reactions that involve liquid handling, one or more controlled temperature incubation steps (including temperature cycling), and a purification step based on molecular size discrimination. Exemplary applications include but are not limited to polymerase chain reaction (PCR), DNA sequencing applications, oligonucleotide ligation, ligase chain reaction (LCR), single nucleotide extension, exonuclease treatment, and oligonucleotide hybridization assays.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

DEVICE FOR RAPID DNA SAMPLE PROCESSING WITH INTEGRATED LIQUID HANDLING, THERMOCYCLING, AND PURIFICATION

Field of the Invention

The invention relates to devices and methods for high speed, low volume automated sample handling of biological samples, which are useful in the field of genomics for a variety of processes, including DNA sequencing, genetic analysis, and gene expression analysis. The invention further relates to devices and methods for setting up and executing assays for high throughput compound screening for pharmaceutical applications.

Background of the Invention

Laboratory automation has played a key role in the advancement of genomics and drug discovery over the past decade. Early work in genomics focused on the automation of fingerprinting and STS mapping procedures through the adaptation of pipetting robots and image acquisition systems (Garcia *et al.*, 1995, Kwok *et al.*, 1992, Lamerdin and Carrano, 1993, MacMurray *et al.*, 1991, Nizetic *et al.*, 1994, Sloan *et al.*, 1993). An interesting example was the "Genomatron" for STS/EST mapping, jointly developed by Intelligent Automation Systems and the Whitehead Institute Genome Center in Cambridge, MA (Dietrich *et al.*, 1995, Hudson *et al.*, 1995). This system performed all the necessary steps for high-speed PCR setup, thermocycling, sample processing for transfer of the reaction products onto nylon membranes, and hybridization with biotinylated probes for CCD based optical signal detection. However, the machine was large, expensive to operate, and could not be easily adapted to performing other tasks. From 1990 onward, a large number and variety of laboratory automation devices became available from an ever expanding set of instrumentation companies. Automated systems are now used in high-throughput sample preparation for DNA sequencing at many of the large sequencing centers.

The degree of automation of the various functions performed in sequencing centers varies widely, ranging from manually fed systems to fully integrated processes. Each extreme has demonstrated positive attributes that contribute to its successful implementation in the sequencing laboratory. However, with a manual setup, there are

significant problems with human error resulting in misidentification of sequencing reads, while the fully integrated process is prone to system failure should one of the modules break.

The current approach in the automation community is to move away from large fully integrated systems to smaller workstations that fulfill specific independent functions in the sequencing process. This means that malfunction of one workstation does not result in a total system breakdown. In addition, this paradigm allows flexibility, which can accommodate changes in requirements as sequencing processes change and improve over time. Specifically, as it takes time to build an automation unit, having flexibility allows one to alter and modify different components as improvements become available. In high throughput sequencing facilities there are several functions which are tedious, inefficient and error prone. Examples of these are colony picking, template preparation, sequencing reaction setup, clone retrieval and gel loading.

Modern laboratories employ partially automated procedures for handling samples. In these procedures, reagents and templates are combined by manually feeding 96-channel pipettors with thermocycling plates. Other laboratories utilize pipetting robots, such as the Tecan Genesis (Ahmadi, 1997) to accomplish the same task. Integrated systems that utilize a variety of pipetting robots, and plate-to-plate liquid transfers, plate sealing, and plate-based thermocycling with magnetic bead or filtration based purification procedures have been constructed. However, these systems are complicated, expensive to build, and suffer from sample evaporation problems and volume constraints.

Another significant drawback in standard 5-10 µl sequencing reactions is that at least 50% of the sample is wasted, never being loaded on the gel. Furthermore, the amount of fluorescently labeled DNA that can be detected on current sequencing machines is much lower than the amounts that are typically processed (0.5-1 µl samples are sufficient).

Several groups have proposed using glass capillaries to handle large numbers of DNA sequencing samples. For example, the first protocols for chemical sequencing developed by Maxam and Gilbert (1977) utilized sealed glass capillaries to handle the samples. In one case, the capillaries are filled, mixed and handled individually as they are moved through several functional "stations" on a conveyor belt type of arrangement

(Friedman and Meldrum, 1998). In another developmental project, 96 capillaries are attached to a Hydra dispenser (Robbins Scientific) so that the samples can be moved up and down past heating elements to perform PCR (Hunicke-Smith, 1997). In a revision of this device, copper heating elements were moved up and down with respect to the position of the samples (Stanford Technology Lab, 1998).

The use of capillaries as sample handling vessels for DNA sequencing has been attempted but, until now, there has been no simple, inexpensive method for accomplishing this purification in very small volumes (< 1μl). Many processes and techniques have been developed to accomplish this task, but the existing techniques have many shortcomings. For example, ethanol precipitation is not fully effective in removing salts and nucleotides and is incapable of removing primers. Furthermore, this technique often results in loss of DNA and is difficult to automate. Commercially available kits such as Quantum Prep ™ SEQueaky Kleen 96 (catalog number 732-6260, Bio-Rad Laboratories, Hercules, CA) and Multiscreen 96 (catalog number MAHVS45, Millipore Corp., Bedford, MA) accomplish the same task as ethanol precipitation, but still do not remove primers, and require a subsequent centrifugation or filtration step. Hence, multiple processing steps are employed in an effort to purify a DNA sample. Effective procedures for complete salt and template removal using spin columns and ultrafiltration have also been reported (Ruiz-Martinez *et al.*, 1998; and Salas-Solano *et al.*, 1998), but the materials are costly and the procedures are not readily amenable to automation.

There exists a need in the art for small-volume DNA purification methods, preferably with re-usable components, that can be easily integrated with capillary-based sample handling, and that eliminate the need for centrifugation. There also exists a need for capillary-based clean-up devices and sample handling alternatives to air-driven thermocycling, and for methods for efficiently handling sample amounts that are just sufficient for each separation, in order to achieve significant cost savings. There also exists a need to minimize sample deterioration and cross-contamination in the aforementioned devices and methods.

Summary of the Invention

The invention relates to an integrated, capillary-based sample handling system for capillary-based aspiration, incubation, purification and delivery of a biological sample that is capable of processing many samples in parallel. The invention further provides integration of pipetting, mixing, temperature treatment, and sample purification that is easy to operate and can be re-used many times.

Thus, in one embodiment, the invention is a device for integrated processing of a biological sample. The device comprises a first capillary tube sized for holding the biological sample, and having a first end and a second end; a second capillary tube sized for holding the biological sample, and also having a first end and a second end; and a connector coupled to the second end of the first capillary tube and the first end of the second capillary tube for mechanically and fluidly connecting the first and second capillary tubes together.

In another embodiment, the device comprises a first capillary tube sized for holding the biological sample, and having a first end and a second end; a first regulating element coupled to the first end of the first capillary tube for selectively providing a fluid seal thereat; a second capillary tube sized for holding the biological sample, and also having a first end and a second end; and a second fluid regulating element disposed between the second end of the first capillary tube and the first end of the second capillary tube for selectively providing a fluid seal between the first and second capillary tubes. In yet another embodiment, the device comprises a first capillary tube sized for holding the biological sample and adapted for thermal processing of the sample, the first capillary tube having a first end and a second end; a second capillary tube sized for holding the biological sample, the second capillary tube also having a first end and a second end; and a fluid regulating element coupled to the second end of the first capillary tube and the first end of the second capillary tube for mechanically and fluidly connecting the first and second capillary tubes together.

In still another embodiment, the invention is a device for integrated processing of a biological microsample. The device comprises a first capillary tube sized for holding the biological microsample and adapted for thermocycling the sample, the first capillary tube having a first end and a second end; a second capillary tube sized for holding the biological sample and adapted for equilibrium dialysis of the sample, the second

capillary tube also having a first end and a second end; and a connector coupled to the second end of the first capillary tube and the first end of the second capillary tube for mechanically and fluidly connecting the first and second capillary tubes together.

Another embodiment of the invention is a device for dialysis of a biological microsample. The device comprises a capillary tube sized for holding the microsample and comprising separation means for purifying the microsample by molecular size discrimination. In a preferred embodiment, the invention is a device for equilibrium dialysis of a biological sample. In another preferred embodiment, the invention is a device for flow dialysis of a biological sample. In yet another preferred embodiment, the invention is a device for exchange dialysis of a biological sample.

In another aspect, the invention is a modular device for integrated handling of biological sample. In one embodiment, the modular device which comprises a first modular array of capillary tubes for holding the biological sample, the first modular array having a first array end and a second array end; a second modular array of capillary tubes for holding the biological sample, the second modular array being coupled to the second array end of the first modular array, wherein the second modular array is removably and replaceably coupled to the first modular array; and an array of fluid regulating elements coupled to the second array end of the first modular array and a first end of the second modular array for selectively providing a fluid seal between the first and second modular arrays of capillary tubes.

In another embodiment, the invention is a modular device for integrated processing of a biological microsample. The device comprises a first modular array of capillary tubes sized for holding the biological microsample and adapted for thermocycling the sample, the first modular array having a first array end and a second array end; a second modular array of capillary tubes sized for holding the biological microsample and adapted for dialysis of the sample, the second modular array also having a first array end and a second array end, wherein the second modular array is coupled to the second array end of the first modular array, and wherein the second modular array is removably and replaceably coupled to the first modular array; and an array of connectors coupled to the second array end of the first modular array and the first end of the second modular array for selectively providing a fluid seal between the first and second modular arrays of capillary tubes.

Another embodiment of the invention is a modular device for dialysis of a biological microsample. The device comprises a modular array of capillary tubes sized for holding the microsample, each of the tubes comprising separation means for purifying the microsample by molecular size discrimination.

In still another aspect, the invention is a system for automatic and integrated processing of a biological sample. In one embodiment, the system comprises a first capillary tube for holding the biological sample, the first capillary tube having a first end and a second end; a support assembly for supporting the capillary tube at its first end; a second capillary tube for holding the biological sample, the second capillary tube also having a first end and a second end; a first fluid regulating element disposed between the second end of the first capillary tube and the first end of the second capillary tube for selectively providing a fluid seal between the first and second capillary tubes; and a plate handling assembly positioned for selectively disposing a fluid reservoir beneath the second capillary tube during operation.

In another embodiment, the system comprises a first capillary tube for holding the biological sample, the first capillary tube having a first end and a second end; a support assembly for supporting the capillary tube at its first end; a first fluid regulating element disposed between the support element and the first end of the first capillary tube for selectively providing a fluid seal between the support element and the first capillary tube; a second capillary tube for holding the biological sample, the second capillary tube also having a first end and a second end; a second fluid regulating element disposed between the second end of the first capillary tube and the first end of the second capillary tube for selectively providing a fluid seal between the first and second capillary tubes; and a plate handling assembly positioned for selectively disposing a fluid reservoir beneath the second capillary tube during operation.

Another aspect of the invention is a modular system for automatically processing a biological sample. The system comprises a first modular array of capillary tubes for holding the biological sample, the first modular array having a first array end and a second array end; a support assembly coupled to the first array end of the first modular array for supporting the array at its first end; a second modular array of capillary tubes for holding the biological sample, the second modular array coupled to the second array end of the first modular array, wherein the second modular array is removably and

replaceably coupled to the first modular array; and a plate handling assembly positioned for selectively disposing a fluid reservoir beneath the second modular array of capillary tubes during operation.

In another aspect, the invention is a method for processing a biological sample. In one embodiment, the method comprises providing a first capillary tube sized for holding the biological sample, providing a second capillary tube sized for holding the biological sample; mechanically and fluidly coupling a second end of the first capillary tube and a first end of the second capillary tube together; supporting the first capillary tube at a first end; and positioning a fluid reservoir beneath the second capillary tube during operation.

In another embodiment, the invention is a method for integrated processing of a biological microsample. The method comprises temperature treating the microsample, and purifying the microsample, wherein the temperature treating and purifying steps are carried out in a device hereinabove described that integrates these steps, such that integrated processing of the microsample is achieved.

In yet another embodiment, the invention is a method of purifying a biological microsample. The method comprises introducing the microsample into a capillary tube which comprises separation means for purifying the sample by molecular size discrimination, and allowing the microsample to reside in the capillary tube for a time sufficient such that purification of the microsample is achieved. In one embodiment, the step of purifying the microsample includes dialysis. In another embodiment, the step of purifying the microsample includes flow dialysis. In another embodiment, the step of purifying the microsample includes exchange dialysis.

In a further embodiment, the invention relates to purifying and cleaning methods that remove contaminants quickly and efficiently from a DNA reaction mix. In a particularly preferred embodiment, the DNA reaction mix comprises DNA templates produced in host cells.

In another embodiment of the invention, the capillaries are designed to prevent leakage, *e.g.*, by sealing, but at the same time to allow penetration, *e.g.*, by a syringe. This arrangement allows for the application of positive or negative pressure. For example, this arrangement permits aspiration of samples from microtiter plates into the capillaries, and subsequent dispensing back into plates for further processing.

In a further embodiment of the invention, multiple samples are thermocycled in a single capillary at the same time.

In another embodiment of the invention, DNA and sequencing reagents are metered and mixed in a single capillary.

In yet another embodiment, the invention provides a guide cap located above the seals at the top and bottom of each capillary tube. This arrangement serves to guide a syringe needle into the opening of the capillary.

In preferred embodiments, the capillary tubes are made of glass, fused silica or TEFLON®.

Brief Description of the Drawings

The foregoing and other objects, features and advantages of the invention will be apparent from the following description and apparent from the accompanying drawings, in which like reference characters refer to the same parts throughout the different views. The drawings illustrate principles of the invention and, although not to scale, show relative dimensions.

FIG. 1 is a schematic perspective view of a capillary based sample handling system in accordance with the teachings of the present invention.

FIG. 2 is a schematic perspective view of one capillary stage for achieving purification of the biological sample of the system of FIG. 1, in accordance with the teachings of the present invention.

FIG. 3 is a schematic perspective of a modular array of capillary tubes suitable for employment with the sample handling system of FIG. 1, in accordance with the teachings of the present invention.

FIG. 4 depicts an electrophoretic gel comparing sequencing reaction clean-up results obtained using an embodiment and method of the present invention (Dialysis) to results obtained using ethanol precipitation, as further described in Example I.

FIG. 5 depicts an electrophoretic gel comparing results obtained using an embodiment and method of the present invention with either ET primer mix or Big Dye Terminator mix as sample, as further described in Example II.

FIG. 6 is a schematic perspective view of a capillary based sample handling system employing a single capillary tube according to the teachings of the present invention.

Detailed Description of the Invention

Definitions

Before further description of the invention, certain terms employed in the specification, examples and appended claims are, for convenience, collected here.

The term "biological sample" refers to a sample comprising one or more cellular or extracellular components of a biological organism. Such components include, but are not limited, to nucleotides (*e.g.*, DNA, RNA, fragments thereof and plasmids), peptides (*e.g.*, structural proteins and fragments thereof, enzymes, *etc.*), carbohydrates, *etc.* The biological samples described herein may also include transport media, biological buffers and other reagents well know in the art for carrying out the processes described above. Although the methods of the invention can be carried out with a biological sample of just about any volume, biological samples in accordance with the invention typically have microliter (μL) volumes and therefore can be referred to as microsamples, *e.g.*, biological microsamples. The methods of the invention are advantageously practiced with biological samples having volumes ranging from 10 μl to 0.05 μL, preferably 0.1 μL to 3 μL.

The term "cassette" refers to a structure or "module" capable of accommodating an array of capillary tubes handling a plurality of samples, *e.g.*, 96 or more samples.

The term "dialysis" is art-recognized and is understood to refer to the separation of substances in solution by means of their unequal diffusion through a membrane. As used herein, "equilibrium dialysis" refers to dialysis which occurs without exchange or flow of dialysate. "Flow dialysis" refers to dialysis which occurs with a flow (or

counterflow) of dialysate. "Exchange dialysis" refers to dialysis which includes at least one change of the dialysate surrounding the membrane.

The term "integrated processing" refers to a process comprising at least two distinct steps which are "integrated" in the sense that the steps are carried out in a single operation. Such a process includes, but is not limited to, template purification, polymerase chain reaction (PCR), DNA sequencing, polynucleotide ligation, cloning, ligase chain reaction (LCR), single nucleotide extension reaction, exonuclease treatment, and oligonucleotide hybridization reactions. Process steps associated with these processes include, for example, the aspiration, mixing, incubation, purification, temperature treating, such as heating or cooling, and delivery of the biological sample alone or in a biologically compatible carrier fluid in a selected manner. The invention described herein integrates these distinct steps; *i.e.*, the invention makes it possible for these steps, heretofore carried out in separate operations, to be carried out in a single operation.

The term "membrane element" refers to a material which may used to separate substances in solution by means of unequal diffusion, *e.g.*, by size exclusion. Exemplary membrane elements are semipermeable; i.e., the membrane elements are capable of permitting dialysis to take place.

The term "purification" is intended to encompass, in its various grammatical forms and synonyms (*e.g.*, purification, purifying, clean up, *etc.*) any operation whereby an undesired component(s) is/are separated from a desired component(s). Such operations include, but are not limited to, filtration, ultrafiltration, dialysis/equilibrium dialysis, chromatography, *etc.* In certain embodiments, purification is achieved by molecular size discrimination among the components of the biological sample. Purification by molecular size discrimination can be achieved using any number of materials of varying porosity well known in the art including, but not limited to, filters, membranes, and semipermeable ultrafiltration fiber materials.

The terms "temperature processing," "temperature treating," and "thermal processing" are used interchangeably herein to refer to the application of a variety of temperature conditions to the sample, depending on the particular process underway and include, but are not limited to, continuous and discontinuous heating regimens, *e.g.*, denaturation, annealing, incubation, precipitation, *etc.* For example, the terms broadly

encompass thermocycling associated with PCR and similar processes. The term "ultrafiltration" refers to any method of dialysis wherein the sample is under positive pressure relative to the dialysate.

The invention described herein includes an integrated capillary-based sample handling system for automated capillary-based processing of the present invention. The system is capable of processing many samples in parallel, if desired, using standard micro-titer plates as reagent sources. The use of capillary tubes in connection with the present invention has the advantage that only a small fraction of the liquid volume is exposed to the atmosphere, so that evaporation is minimized. This promotes the processing of the sample, while concomitantly eliminating or reducing sample loss. The capillary tubes of the system can be used to retrieve, mix and dispense fluids by integration with air or liquid-filled volumetric devices, such as piezoelectric elements, movable pistons or syringe-type plungers.

Figure 1 is a schematic depiction of the capillary based sample handling system 10 in accordance with the teachings of the present invention. The illustrated system 10 employs a support assembly 12 that is configured for supporting and/or mounting a capillary based processing assembly 14. The support assembly 12 positions the capillary based processing assembly 14 over a platen surface 30 that is sized and dimensioned for supporting a reservoir plate 32. A controller 36 is coupled to selected components of the sample handling system 10, such as the support assembly and various portions of the processing assembly 14, as well as to a temperature regulating source 40. The support assembly 12, platen 30, and control 36 can form part of a conventional fluid dispensing unit, such as a Hydra dispenser, manufactured by Robbins Scientific, U.S.A., and which can be modified in an appropriate manner obvious to the ordinarily skilled artisan in light of the teachings herein to operatively mount the capillary based processing assembly 14. Those of ordinary skill will also recognize that other fluid dispensing and sample handling units presently employed and in practice, whether in modular or discrete forms, can be employed to perform the features and functions described herein, provided they are adapted to mount the capillary based processing assembly 14 of the present invention.

The illustrated processing assembly 14 includes multiple, discrete components that are functionally and operatively connected in a selected manner so as to perform multiple processing steps upon a biological sample. The capillary based processing assembly 10 includes multiple, operatively connected capillary tubes that are coupled in a selected fashion to perform multiple processing steps in a single, vertically integrated unit. The illustrated assembly 14 includes a fluid coupling 44 that is connected at one end to the support assembly 12, and at an opposite end to a fluid regulating device 48. The fluid coupling 44 operates in connection with the controller 36 and support assembly 12 to regulate fluid movement within the processing assembly 14. For example, the fluid coupling 44 is coupled to a pump mechanism, which forms part of the support assembly 12, to either introduce fluid into or discharge fluid from a proximal end of the processing assembly 14. Those of ordinary skill in the art will readily recognize that the fluid coupling 44 can comprise any suitable self contained fluid actuating mechanism, such as a plunger-type or a syringe-type mechanical coupling, or can be any other suitable fluid conduit that serves as a fluid and mechanical coupling between the support assembly 12, which can comprise a fluid movement or pump mechanism, and the remainder of the processing assembly 14. The fluid coupling can also optionally include a guide for a syringe/syringe needle at the top and/or bottom of the capillary tube 50.

With further reference to Figure 1, the illustrated fluid regulating device 48 can be a valve-type structure, such as a fluid valve or pinchable silicone connector, that is adapted for selectively placing the fluid coupling 44 and selected fluid communication with the remainder of the processing system, such as the capillary tube 50. The fluid regulating device 48 can either be operated manually, or can be part of an overall automated sample handling system, such as the automated sample handling system 10 of the present invention, by coupling via any suitable communication pathway to the illustrated controller 36. The capillary tube 50 can be a commercially standard and available capillary element that is sized and dimensioned for fluidly retaining microliters of a biological fluid. According to a preferred practice, the illustrated capillary tube 50 is adapted for holding a biological sample in a biologically compatible carrier medium. Examples of suitable biological samples include DNA, proteins and other like biological

components, and the biologically compatible carrier medium can be any known buffer or processing fluid typically employed in biological processing techniques.

The illustrated capillary tube 50 is further coupled at a proximal end 50A to a second fluid regulating device 48. The fluid regulating device 48 is preferably similar to the fluid regulating device coupled to the distal end 50B of the capillary tube 50. The combination of the pair of fluid regulating devices 48, (48 coupled to either end of the tube 50 can fluidly isolate the chamber of the capillary tube 50 from the remainder of the processing system 14). This configuration allows a selected processing regimen as implemented by the controller or by hand to occur within the confines of the capillary tube independent of any other activities being performed in any other section of the processing assembly 14. For example, a temperature regulating source 40, which can include a heating element or a cooling source, is thermally coupled to the capillary tube 50 when the processing assembly 14 is disposed in a selected position. The temperature regulating source 40 is preferably a heating source that heats the capillary tube 50. In this manner, the biological sample and associated carrier fluid, if desired and contained within the chamber of the capillary tube 50, can undergo a thermocycling process according to a user selected regimen. Those of ordinary skill will readily recognize that the temperature regulating source 40 can comprise a series of resistive heating elements, or can be a heating source that passes heated air across the outer surface of the capillary tube 50.

The illustrated capillary tube 50 can be made of glass, or is coated with a polyimide coated fused silica. Suitable capillary tubes for use in accordance with the teaching of the present invention are within the purview of one of ordinary skill, when considering the particular temperature ranges, capillary size, heating duration, and specific contents and quantities of the biological sample and carrier fluid. The ordinarily skilled artisan in light of all these parameters would be able to determine the appropriate capillary tube size and length. Moreover, the particular temperatures in which to heat the contents of the capillary tube are also within the purview of one of ordinary skill when considering the particular type of processing that is desired to be carried out within the capillary tube, and the type and quantity of the biological sample and carrier fluid.

With reference again to Figure 1, a second capillary tube 52 has a distal end 52A coupled to the intermediate fluid regulating device 48, and has a proximal end 52B coupled to a third fluid regulating device 48. As set forth above, the fluid regulating devices 48, coupled to the proximal and distal ends 52A and 52B of the capillary tube 52, also serve to isolate fluidly the capillary tube 52. Consequently, a second or additional processing regimen can occur within the capillary tube 52 independently, contemporaneously and concurrently of any particular processing regimen that occurs within the other capillary tube 50.

A fluid tip 56 having a central lumen is coupled to the third fluid regulating device 48 in accordance with known techniques. The fluid tip 56 can serve to either transfer fluid to or receive fluid from the reservoir 32 disposed on the platen 30 of the sample handling system 10. The illustrated reservoir 32 can be a standard 96-well micro-titer plate, and hence can be employed in connection with the illustrated system 10 to perform multiple, parallel processing of biological samples. The fluid tip is preferably any suitable mechanical coupling that fluidly connects the processing assembly with an external fluid source, such as the fluid reservoir, according to one mode of operation. The tip is preferably TEFLON® coated, and can be made of plastic or stainless steel. Other types of tips obvious to the ordinarily skilled artisan can also be used.

The illustrated processing assembly 14 provides for a single, stacked, and integrated processing sub-assembly 14 that allows for single or multiple biological samples to be aspirated throughout the axial length of the assembly, and can isolate, in connection with the fluid regulating devices, selected portions of the assembly to perform different processing regiments in parallel.

The illustrated control 36 is programmed to control, according to user selected information, the fluid regulating elements 48 to selectively connect or disconnect either of the capillary tubes 50 and 52 relative to each other. For example, the controller 36 can dispose in an open position the illustrated fluid regulating devices 48 to effectively form a single axial fluid lumen along about the entire axial length of the assembly 14; that is, from the support assembly to the exit port of the fluid tip 56. Alternatively, the controller 36 can actuate one or more of the fluid regulating devices 48 to fluidly separate or isolate one or more regions of the processing assembly from the remaining

regions. The illustrated controller 36 also controls the support assembly 12. According to a preferred practice, the controller provides selected control data that instructs the support assembly 12 to position the processing assembly 14 at selected positions to either retrieve or introduce fluid to and from the reservoir 32. The axial movement imparted by the support assembly 12 to the processing assembly 14 is indicated by the arrow 58. The illustrated controller 36 further provides control signals to the temperature regulating source 40 to selectively actuate the source to provide heating or cooling of a particular region of the processing assembly 14. Although the illustrated temperature regulating source 40 is positioned for regulating the temperature of the first capillary tube 50, those of ordinary skill will readily recognize that the support assembly 12 can position other portions of the processing assembly 14, such as the second capillary tube 52, in a position to be heated or cooled by the source 40. Alternatively, multiple sources 40 can be used. Moreover, the illustrated support assembly 12 and/or controller can move the processing assembly 14 in the horizontal direction, as indicated by arrow 58A.

A significant advantage of the illustrated sample handling system 10 is that it provides for an automated, high speed sample handling system that can process low volumes of a biological sample in an automated format, without the need for centrifugation which typically requires large volumes of sample. Moreover, the illustrated processing assembly of the system 10 preferably employs reusable components, thereby significantly extending the useful life of the major system components. The integrated processing assembly 14 also provides for an efficient, compact and relatively simple processing assembly for performing one or more processing regimens in parallel, and if desired, independently of each other.

A significant advantage of employing multiple capillary tubes in the processing assembly 14 is that the sample volumes provided by each capillary tube allows the processing of significantly smaller sample portions, since relatively small volumes of the overall carrier fluid disposed within the capillary tubes are subject to evaporation. This sample conservation advantage significantly reduces the sample volumes necessary to achieve selected processing of the sample, while concomitantly affording sample outputs that have sequencing ladders with improved signal strength and resolution.

According to a preferred practice, the capillary tubes preferably have internal volumes that accommodate fluid sizes of less than about 1 microliter.

An advantage of employing the novel submicroliter capillary tubes, in tandem, as illustrated in Figure 1, is that it allows the use of minimal amounts of expensive sequencing reagents and relatively small volumes of biological samples in an automated sample handling format. The illustrated processing assembly 14 can be used to perform purification procedures on polymerase chain reaction (PCR) products, preparing sequencing ladders, and injecting the sequencing ladders into appropriate microtiter plates, or aspirating the biological products into particular zones of the processing assembly 14. The particular processing regimens which can be accommodated or performed within particular zones of the processing assembly 14 are described in detail below.

According to one practice, the illustrated processing assembly 14 can be employed to purify a biological sample disposed within one or more regions of the processing assembly. For example, as illustrated in Figure 2, the capillary tube 52, which is coupled to a fluid regulating device 48 at either end, can house a separation element, such as a semipermeable microfiber, for processing a biological sample, such as DNA. According to one technique, the capillary 52 and illustrated microfiber 60 can be employed to perform equilibrium dialysis within the confines of a capillary electrophoretic system. Typically, DNA sequencing products are purified to remove excess salt, nucleotides, primers, and templates from the biological sample. The illustrated microfiber 16 can be employed to perform the filtration process upon the DNA, to exclude the desired products, while concomitantly allowing undesired components to pass therethrough when the processing assembly is exposed to a pressure or vacuum condition at a proximal end. The DNA sample is cycled through the microfiber by the pressure formed within the system, thereby resulting in relatively small components being filtered out of the hollow fibers and hence the sample. The use of a capillary tube with one or more microfibers disposed therein, provides for the ability to perform equilibrium dialysis upon very small volumes of between about 10 to 0.05 microliters.

As illustrated in Figure 3, an additional significant advantage of the present invention is that the capillary tubes 50 and 52 can form part of an array of capillary tubes 70, *e.g.*, a cassette of capillary tubes. The illustrated array of capillary tubes allow the illustrated sample handling system 10 to perform multiple, parallel processes in a vast array of processing assemblies 14. In particular, the system 10 can accommodate the use of multiple arrays of the capillary tubes 50 and 52. For example, the capillary tubes 50 can comprise part of an array, and the capillary tube 52 can also form part of a second array of capillary tubes. The illustrated fluid regulating devices 48 can be interposed between the modular capillary tube arrays, to form a modular and stackable assembly of processing tubes for use in connection with the illustrated system 10. The illustrated controller 36 can provide instruction data to a suitable robotic assembly, such as a pipetting robot, to selectively stack together or remove one or more of the modular arrays of capillary tubes 50 and 52 from the processing assembly 14 of the illustrated system 10. This modular aspect of the illustrated system 10 allows for the removable and replaceable use of modular arrays of capillary tubes, in order to aspirate or introduce biological samples to a standard 96-well microtiter plate. Moreover, the stackable aspect of the modular array of capillary tubes allows multiple selected processes to be conducted within one or more of the capillary tubes. Upon completion of the processing regimen, the system can disconnect one or more of the modular arrays with the pipetting robot for downstream processing or storage.

In accordance with another embodiment of the present invention illustrated in Figure 6, the sample handling system 10' can employ a single capillary tube 51 for processing a biological sample. The sample handling system 10' can be used in applications in which it may be desirable to effect a single processing regimen upon the biological sample. Such processing regimens can include sample purification and thermocycling, as well as any other of the sample processing steps described herein. In the case of sample purification, a membrane element, such as the microfiber 60 illustrated in Figure 2, can be provided within the capillary tube 51.

According to the invention, purification of a sample may be achieved by a variety of methods, including dialysis, filtration, ultrafiltration and chromatography. The invention further provides various configurations to achieve purification, depending on the method of purification selected. For example, when equilibrium dialysis is the

method of purification, the apparatus of the invention provides at least one capillary comprising a membrane element in operative contact with a dialysate, *e.g.*, water. In certain embodiments, the dialysate is contained in a cassette. When exchange dialysis is the method of purification method, the capillary may be inserted successively into at least two cassettes containing a dialysate.

As set forth herein, the present invention includes dialysis techniques, which may be used effectively to "clean up" polymerase chain reaction (PCR) and cycle sequencing reactions. Until now, one of the problems with conventional dialysis techniques has been one of scale. Typically, dialysis is carried out on relatively large sample volumes of at least 1 mL or more. The typical PCR or sequencing reaction, on the other hand, generally utilizes sample volumes of approximately 10 μL or less, significantly smaller than the sample volumes in conventional dialysis techniques.

The present invention addresses this disparity by using a membrane element, such as one or more microfibers inserted within one of the capillary tubes. The microfiber performs the same separation functions as the much larger dialysis operations, but with much smaller sample volumes and without the use of centrifugation. The microfibers can be generated or manufactured by removing one or more hollow fibers from commercially available filtration cartridges. Typical cartridges contain many hundreds of fibers, since the cartridge is solely designed to perform dialysis on large sample volumes, *e.g.*, 1 mL or more. Many types and sizes of hollow fiber filtration cartridges are available through such suppliers as Millipore Corp. Bedford, MA or Spectrum Labs Laguna Hills, CA. Typically these cartridges are used as ultrafiltration devices, where the dialysis membrane acts as a filter, excluding the desired products while allowing the undesired components to pass through when pressure or vacuum is applied to the system. The present invention achieves proper filtration or separation of components from small volumes of a biological sample by employing one fiber for each biological sample. In this way, dialysis on sample volumes of 10 to 0.05 μL volumes is achieved.

According to one mode of operation, the present invention achieves appropriate purification of a sample by first performing a standard *Big Dye Terminator Cycle Sequencing Ready Reaction Kit*, part # 4303154 PE Applied Biosystems Foster City CA, on a reaction sample size of between 0.05-10 μl. The sample volume is drawn up into a

hollow fiber filter which has been cut out of a Spectrum cartridge cat # 132229 Spectrum Labs Laguna Hills, CA using a 10 µl syringe from Hamilton, Reno, Nevada (see FIG. 2). Purification is then achieved according to any of the various methods described herein.

One advantage of the present invention is the integration of pipetting, mixing, temperature control/thermocycling, and sample purification in a simple, single integrated flow-through system 10 (or processing assembly 14) that is easy to operate and can be reused a significant number of times. Sample components are aspirated and mixed on a fluid tip conduit 56, such as a TEFLON® coated tip, TEFLON® tubing, other plastic type tubing, stainless steel, or TEFLON® coated stainless. Thermocycling is achieved, for example, by blowing air of different temperatures over a capillary tube 50 made of glass, or more preferably, polyimide coated fused silica, although a liquid medium could also be used for heat transfer. Capillary ultrafiltration material allows the removal of unwanted reaction components that are small in molecular size compared to the desired DNA products by dialysis against water in an adjacent chamber.

The technique of dialysis, although well established, has heretofore been difficult to perform on small sample volumes without suffering loss of the sample. Semi-permeable microfiber ultrafiltration materials are available in a variety of porosities, which allow small components to freely pass through while larger components are selectively retained. Although these are commonly used for ultrafiltration of proteins, only some of the materials are suitable for capillary-based dialysis. Because all of the reaction components to be removed from PCR and DNA sequencing reactions are much smaller than the desirable products, the process of the present invention is an optimal method to "clean up" these reactions.

According to one embodiment of the present invention, each zone of, or processing regimen performed by, the sample handling device (*e.g.*, mixing, cleaning, thermocycling, and liquid handling) can be isolated from the others by the illustrated valves 48 to prevent 'wandering' of the sample during each step of the procedure. A linear or rectangular array of such devices interfaces conveniently with a microtiter plate 32 disposed on the system platen 30. The dialysis and thermocycling sections of the array are enclosed within separate sealed containers to provide controlled liquid or air flow across the devices.

This arrangement allows sub-microliter quantities of liquids to be aspirated by negative pressure on the system. The samples are specifically moved to various parts of the system by controlled pressure changes. Note that in a capillary of diameter 250 μM, a volume of 0.5 μl occupies a height of approximately 1 cm. A number of commercial devices are available on the market that may be adapted in accordance with the teachings of the present invention for small volume aspiration and delivery utilizing capillary sample holders. These include syringe pumps from several manufacturers, 96- and 384- channel sample liquid dispensers from Robbins Scientific, Inc. (Hydras), "Nanomovers" from Precision Scientific, and several other possible designs. The Hydra systems are convenient because of the ability to simultaneously, accurately, and coherently aspirate and deliver selected volumes from parallel channels. These systems offer ease of integration with physical plate-handling systems and PC-based programming systems through an RS232 port.

For temperature control and thermocycling, a two-temperature air-circulation system with appropriately placed valves may be used to enable a wide range of air temperatures to be quickly attained. For example, the heating source 40 can be employed to heat a sample disposed in the capillary tube 50 and isolated from other sections of the processing assembly 14 by a pair of fluid regulating devices 48.

Thus, in one embodiment, the thermocycler will use a combination of hot and cold air to change sample temperature. Simple air blowers or blowing ambient air and air heated by resistance heaters over the capillaries may be used to change the temperature. The temperature may be measured and controlled by standard PID controllers. The heating rate may be increased as desired by using, for example, superheated air for the first part of the heating cycle, then cooler air to avoid excessive overshoot of the temperature of the capillaries.

The in-line processing assembly 14 mounted to the support 12 of the sample handling system 10 integrates sample aspiration, mixing, thermocycling, dialysis and dispensing in a single device. This achieves definite advantages in terms of sample integrity (no evaporation), simplicity and throughput. Optical sensors may be employed in connection with the illustrated system 10 to detect liquid levels at one or more points in the system 10, and provide open loop or feedback control to the controller 36 to adjust, if necessary, the sample or fluid level volumes within one or more sections of the

processing assembly. The present system 10 provides an accurate volumetric system that is much simpler to operate and maintain than conventional systems. For accurate aspiration of small liquid volumes, it is advantageous to maintain a minimal air gap (water can fill almost the entire system) and to coat the outside surface of the capillaries with a hydrophobic material such as TEFLON® to avoid adherent drops and carry-over. Thus, volumes can be accurately aspirated and dispensed at the tip 56, and moved to precise positions within the system. Different solutions (for instance, DNA and *Big Dye Terminator Cycle Sequencing Ready Reaction Mix* (DT-mix)) can be aspirated in separate "slugs" with a small air gap in between (with minimal cross-contamination). When the samples are dispensed back to the tips, the liquid droplet (provided it is not too large relative to the size of the tip) adheres to the tip causing the samples to mix. The droplet can then be aspirated back into the system. Silica capillaries are available with consistent dimensions to within 2 microns.

The invention also relates to purifying and cleaning methods that remove contaminants quickly and efficiently from a DNA reaction mix. Current sequencing machines use electrophoresis through a gel to separate and detect different lengths of DNA that have been appropriately labeled. To make these machines provide results faster and more accurately, the shapes of the gel separation media have gone from thick gels to a gel captured by thin capillaries. A major drawback is the contaminants in the DNA being sequenced tend to physically plug the capillary and interfere with the accurate detection of the different DNA lengths. One major source of contaminants in the DNA sample is the result of by-products of the thermocycling reaction that generates the DNA sample. Both regular and dye-labeled nucleotides that are not incorporated into the DNA strings during the reaction become contaminants that degrade the DNA sequencer. Additionally, ionic components of the reaction reagents remaining in the reaction (*e.g.*, salts) also degrade the machines.

In one embodiment, the present invention provides for effective removal of contaminants from a thermocycling reaction. Once the reaction mixture is thermocycled, purification may be achieved by placing the mixture into a hollow membrane element, which is in contact with a solution having a lower concentration of ionic components. The difference in osmotic pressure across the membrane forces

contaminants in the product to migrate across the membrane into the aqueous solution, effectively removing them from the product.

In another embodiment, the invention provides an apparatus and method for purifying DNA molecules produced in host cells. As further described in Example IV, the invention provides an apparatus and method for producing template DNA in a host cell, lysing the host cell, purifying and sequencing the template DNA.

Exemplification

EXAMPLE I: DNA Sequencing

With reference to Figure 1, the system 14 is first rinsed with water, and the syringes are then filled with water except for about 5 μL. Next , a 1 cm (0.5 μL) air gap is drawn up, followed by 0.5 μl DNA (50 ng/μL), and 0.5 μL of *Big Dye Terminator Cycle Sequencing Ready Reaction Mix* ("DT")(part number 4303154, PE Applied Biosystems, Foster City, CA). DT and DNA are then ejected to mix. 1 μl of the reaction mixture is aspirated and moved up to the thermocycling zone 50 (maintaining a 1-2 cm air gap), and then thermocycling is performed. The sample is moved down to the dialysis zone 52 and dialyzed for 20 min. Finally, the samples are ejected into a microtiter plate and rinsed with water.

During this procedure, a plate handler below changes plates (from DNA source, to DT mix, to output plate) and moves them up and down (to create air gaps). The pipetting, flow-through purification, and thermocycling are fast, so the throughput is very high; *e.g.*, on the order of 384 samples per hour for a 384 channel system. If gels can be run at a rate of 96 samples every 3h (the maximum proposed speed), 1 sample robot could feed 8 gel systems running at full speed.

The sample handling device may also be constructed as a series of independent modules that can be stacked together, or independently attached temporarily to a liquid handling device to effect the liquid handling steps. An advantage of this approach is that a single liquid handling device, which is the most expensive part of the system to build, could be more optimally utilized to process samples in a large number of dialysis and thermocycling modules. The latter modules can then be handled in a similar fashion

as plates are handled on a typical integrated automation system, such that the independent units would be advantageously sealed automatically when detached from the liquid handling device, and that plate-to-plate transfers would be replaced by capillary-to-capillary transfers (avoiding contact with the atmosphere). An example of a dialysis module is shown in Figure 3.

EXAMPLE II: Dialysis

This example further describes the feasibility of using hollow fiber dialysis on very small volumes as a method for sequencing, and PCR reaction clean-up. Most of the work was accomplished on sequencing reactions prepared using PE Applied Biosystems standard *Big Dye Terminator Cycle Sequencing Ready Reaction Kit*, part # 4303154, following the standard 1/4X BigDye Terminator Hydra Sequencing Reactions protocol. The results were obtained on an ABI 377 automated DNA sequencer (PE Applied Biosystems Foster City, CA) or a Megabace 1000 automated DNA sequencer (Molecular Dynamics Sunnyvale, CA). The raw data was analyzed by Phred software (Brent Ewing, LaDeana Hillier, Michael C. Wendl and Phil Green *Base-Calling of Automated Sequencer Traces Using Phred I. Accuracy Assessment* Genome Research 8, pg 175-185; Brent Ewing, Phil Green *Base-Calling of Automated Sequencer Traces Using Phred II. Error Probabilities* Genome Research 8, pg 186-194).

The individual hollow fiber dialysis tubes were obtained by cutting open hollow fiber filtration cartridges produced by Amicon of Beverly, MA and then removing the fibers. Fibers were stored in sterile distilled water until ready for use. Fibers containing different pore sizes were used in these studies but the bulk of the experiments were carried out using filters with an average pore size of 100Kdal.

Standard 1/4X BigDye Terminator Hydra Sequencing Reactions were transferred to the hollow fiber dialysis tubes using a Hamilton syringe (Hamilton Company, Reno, Nevada). The ends of the hollow fibers were closed off by pinching with tweezers. The reactions enclosed by the dialysis fibers were then suspended in a beaker of sterile distilled water and allowed to equilibrate for 30 min. After 30 min the fibers were removed from the beaker, the pinched ends were cut and the contents of the hollow fiber

removed using the Hamilton syringe. Samples were then run on ABI 377s or a Megabace 1000 automated DNA sequencer.

The typical method of removing by-products of the sequencing reaction referred to as "cleaning up" is ethanol precipitation (EtOH prec.). The method of the present invention was compared to EtOH prec. As is evident from Figure 4, visual inspection of a 377 gel comparing results obtained with the dialysis procedures of the invention versus a standard EtOH prec. protocol that the dialysis methodology removes the unincorporated dye terminators which are by-products of sequencing reactions and produces better resolution compared to EtOH precip. A summary of the data is presented in Table 1. Column one describes the readlength which is the total number of reliable bases produced for that particular sequencing reaction. Columns two and three list the total number of bases which have quality scores that are greater than or equal to the Phred scores 30 and 20, respectively. The increase number of bases for the readlengths for dialysis coupled with the increased number of bases have a higher quality Phred scores indicates that the dialysis method produces superior results.

TABLE 1

	Readlength	Phred >30	Phred >20
Dialysis	797	438	602
EtOH prec	637	314	463

EXAMPLE III: Dialysis Kinetics

The following experiment demonstrates the kinetics of the dialysis reaction. Ten microliters of solution containing either BD terminator mix or ET primer mix was used as sample. The BD terminator mix simulates the excess fluorescently labeled dideoxy-terminator products found in a standard sequencing reaction, whereas the ET primer mix simulates the unincorporated primers found in a PCR reaction. Each sample was dialyzed using the above methods with a fibers having an average pore size of 100Kdal for the following time points: 0, 5,10, 15, 30, 60, 120 minutes and one final point at 12 hours or overnight (o/n). The results, as shown in Figure 5, clearly indicate that for the sequencing reactions (*i.e.*, BD terminator mix) dialysis occurs very quickly: equilibrium

occurs within 5 to 10 min. Primers (*i.e.*, ET primer mix) which are much larger, take quite a bit longer to be removed, and in this experiment it took at least 60 min before equilibrium was reached. It should be noted that for the application of primer removal a hollow fiber with a larger pore size, *e.g.*, 500Kdal, is advantageously used.

EXAMPLE IV: Plasmid Purification

Plasmid template purification was performed using 1.1 mm ID diameter tubing having a pore size of 100Kdal. Plasmid subclones containing human BAC inserts were grown in 96 well format and processed by centrifugation, heating at >95°C for 2 min in STET buffer with lysozyme (75uL), and filtration. The cleared filtrate (~30 μL) was then dialyzed for 30 minutes against 1 mM EDTA. Four microliters of the resulting samples were then added directly to Big Dye terminator sequencing mix and the samples were separated on an ABI 377 automated DNA sequencer (PE Applied Biosystems, Foster City, CA). The results from 286 samples processed in this way gave an average clipped readlength of 703 nt and Phred-20 scores of 571 on passed sequences (with an overall pass rate of 85%). These results were essentially indistinguishable from those obtained using a standard automated plasmid preparation with quantification and reconfiguration in accordance with a modified Plasmid Minipreparation Protocol (Millipore Corp., Bedford, MA).

EXAMPLE V: Capillary-based Clean-up and Sample Handling

RapidCycler 1605 (Idaho Technology; Idaho Falls, ID) was tested for small volume thermocycling using glass, silica, TEFLON®, and stainless steel capillaries. Because heat transfer occurs through a low heat capacity medium (air) which can change temperature very quickly, PCR amplification can be accomplished in 30 minutes or less on this system. Sequencing experiments using with Big Dye Terminator chemistry and pGEM-3Zf(+) showed that reagent quantities as low as 1/40 of a standard 20 μl reaction (carried out in 1 μl volumes in glass, silica, or TEFLON® capillaries) could produce sequences of respectable quality and read length. Reactions carried out at

1/20 scale (2 μl) were quite robust and produced high quality reads (Phred-30 scores are shown in the accompanying table 2).

TABLE 2

Thermocyler Condition	Phred-30 score (unpurified sample)	Phred-30 score (purified sample)[c]
[a]Standard Thermocyling Reaction	358	425
[b]Fused Silica Capillary-silanized	285	478
[b]Fused Silica Capillary	433	422

[a] MJ Research Thermocycler, 2 μl reaction volume

[b] Fused Silica Capillaries: I.D.= 250μm (DB-1, J & W Scientific), 2 μl reaction vol.

[c] samples were purified on centri-sep columns

EXAMPLE VI: Capillary Sealing

Visual examination (using a microscope) of 100 nanoliter samples being heated inside a capillary revealed rapid sample dispersal unless both ends of the capillary were sealed (presumably due to rapid outgassing or localized boiling). In experiments with larger sample volumes where only one end of the tubing was effectively sealed by a syringe, the sample slug was observed to rapidly migrate back and forth in the tubing during thermocycling. This was assumed to be a result of the changing vapor pressure in the closed air space within the capillary tubing. The application of slight pressure to the open end of the tubing eliminated the movement and allowed successful thermocycling to be achieved in long TEFLON® capillary tubes. Therefore, sealing means, valves, or a pressure control system are a desirable part of a robust flow-through capillary thermocycling system.

EXAMPLE VII: Thermocycling Several Samples in a Single Capillary

Several samples were thermocycled at the same time in a single capillary. Samples were separated by means of air gaps within the capillaries. As long as the ends of the capillaries were sealed, the samples did not disperse and remained separate from each other. In one experiment, a capillary was loaded with four one-microliter samples,

each with a different dye primer reagent mix. These were thermocycled, pooled, and run on an ABI 377 automated DNA Sequencer (PE Applied Biosystems, Foster City, CA). The results showed that the four primer reactions were successful, and that they did not mix together during thermocycling.

EXAMPLE VIII: Mixing of Reagents

In one embodiment of the invention, sequencing reagents are advantageously added to the DNA prior to thermocycling in accordance with the reaction set up protocol. Traditionally, the dye is metered into a Microtiter plate, then the DNA is metered into the same plate. The plate is then mixed and thermocycled. In accordance with the invention, microliter quantities are thermocycled in a capillary. However it is difficult to aspirate this amount from a microtiter plate, such that it is desirable to include a Dye mix and DNA metering and mixing capability within the capillary. Tests were completed to show that metering and mixing of DNA and sequencing reagent can take place where the reagent is first taken up in a small capillary, and the end touched off, or rinsed, to reduce the amount of extra mix held up on the outside of the capillary. Next, the capillary was immersed into the DNA, and some DNA aspirated into the capillary. This caused very little loss of mix into the DNA source plate. As the DNA and reagent were further aspirated up the capillary, full mixing took place, allowing the sample to be successfully thermocycled. An alternative process was tested to pick up separate slugs of DNA and reagent mix separated by a small air gap and to push these out to the end of the pipette tip without touch-off. With an appropriate tip design (0.5 mm ID TEFLON® capillary tubing, for example) and liquid volume (2 microliters) the microdroplets adhered to the tip and mixed well in the process of coalescence and re-aspiration.

EXAMPLE IX: Sensitivity Testing

A test of overall sensitivity as well as the ability to recover small amounts of DNA from the dialysis tubing was performed as follows. A standard 1/4x Big Dye terminator reaction was carried out, and diluted to the equivalent of 1/32x, 1/64x, and

1/128x. Eleven replicates of the diluted samples were then dialyzed as described in Example II and separated on a MegaBace 100 automated DNA sequencer (Molecular Dynamics Corp., Sunnyvale, CA). The results indicated that the small amounts of sequencing reaction products present in these samples were effectively recovered in approximately 90% of the samples. The readlength and quality statistics are shown in the Table 3 (average of all samples).

TABLE 3

Dilution	Readlength	Phred-20	Phred-30
1/32x	601	526	460
1/64x	634	463	391
1/128x	562	463	383

References

Maxam AM, *et al.* (1977) A new method for sequencing DNA. Proc Natl Acad Sci U S A. 74: 560-4.

Bashkin, J., Roach, D., Leong, J., Bartosiewicz, M., Barker, D., Johnston, R.G. J. (1996) Capillary Electrophor. 3: 61-68.

Boddy, AV; Idle, JR. (1993) Cancer Surv., 17 (Pharmacokinetics and Cancer Chemotherapy), 79-104.

Boffa L.C., Carpaneto E.M. & Allfrey V.G., (1995).*Proc. Natl. Acad. Sci. USA* **92**, 1901

Caporaso, N.; Landi, M.T. (1995) Med. Lav., 86, 199-206.

Carrilho, E; Ruiz-Martinez, M., Berka, J., Smirnov, L., Goetzinger, W., Miller, A.w., Brady, D., Karger, B.L. (1996) Anal. Chem. 68: 3305-3313.

Dubiley S., Kirillov E., Lysov Y. & Mirzabekov A., (1997).*Nucleic Acid Res.* **25**, 2259

Carrilho, E., Miller, A., Ruiz Martinez, M.C., Kotler, L., Keisilman, j., Karger, B.L. (1997) B. L. Proc. SPIE 2985A, 4-18.

Egholm M., Buchard O., Christensen L., Behrens C., Freier S.M., Driver D.A., Berg R., Kim S.K., Norden B., & Nielsen P.E., (1993). *Nature* **365**, 566

Evensen, H. T.; Meldrum, D. R.; Cunningham, D. L. (1998) Rev. Sci. Instrum, 519-526.

Figeys D., Ahmadzedeh H., Arriaga E. & Dovichi N.J., (1996) *J. Chromatogr. A* **698**, 375.

Gingeras, TA.; Mack, D; Chee, MS.; Berno, AJ.; Stryer, L; Ghandour, G; Wang, C. PCT

Int. Appl., 132 pp. WO 9729212 A1 970814.

Haff L., Atwood J.G., . DiCesareJ, Katz E., Picozza E., Williams J.F. & Woudenberg T., *BioTechniques* **10**, 102 (1991).

Meldrum D., *et al.*, (1998) http://isdl.ee.washington.edu/GNL/acapella/

Hacia, JG.; Makalowski, W; Edgemon, K; Erdos, M R.; Robbins, CM.; Fodor, SPA.; Brody, LC.; Collins, FS. (1998) Nat. Genet., 18, 155-158.

Hacia, JG.; Brody, LC.; Chee, MS.; Fodor, SPA.; Collins, FS. (1996) Nat. Genet., 14, 441-447.

Hunicke-Smith, S.P. PCT Int. Appl., 42 pp. Application: WO 97-US10365 970616.

Hunicke-Smith, S.P. (1997) Ph.D. Dissertation, Stanford Univ., Stanford, CA, USA.

Witter C.T. & Garling D.J., *BioTechniques* **10**, 76 (1991).

Linder, M W.; Prough, R A.; Valdes, R, Jr. (1997) Clin. Chem., 43, 254-266.

Masson, E; Zamboni, WC. Can. (1997) Clin. Pharmacokinet., 32, 324-343.

Nickerson, D.A., Tobe, V.O., Taylor S.L. (1997) Nucleic Acids Res. 25: 2745-2751.

Nielsen P.E., Egholm M., Berg R.H., Buchard O., (1991).*Science* **254**, 1497

Parinov S., Barsky V., Yershov G., Kirillov E., Timofeev E., Belgovskiy A. & Mirzabekov A., (1996).*Nucleic Acid Res.* 24, 2998

PerSeptive Biosystem manual.

Rose D.J., *Anal. Chem* **65**, 3545 (1993).

Ruiz-Martinez, M.C., Salas-Solano O., Carrilho, E., Kotler, L., Karger, B.L. (1998) Anal. Chem. 70.

Salas-Solano O., Ruiz-Martinez, M.C., Carrilho, E., Kotler, L., Karger, B.L. (1998) Anal. Chem. 70.

Seeger C., Batz H.G. & Orum H., (1997). *BioTechniques* **23**, 512

Smith, D.R., Doucette-Stamm, L.A., Deloughery, C., *et al.*, (1997) J.Bact.179, 7135-55.

Stanford Technology Lab, (1998) http://sequence-www.stanford.edu/group/techdev/therm.htm

Swerdlow, H., Jones, B., Witter, C.T. (1997) Anal. Chem. 69: 848-855

Tan, H., Yeung, E.S. (1997) Analytical Chem. 69: 664-674.

Tan S.S. & Weis J.H., (1992) *PCR Methods and Applications*: CSH Laboratory Press, 137

Wang J., *J.Amer.Chem.Soc.* **118**, 7667 (1996).

Wang, K. Gan, L., Boysen C. & Hood, L. (1995).Anal. Biochem. 226, 85

Wang R., Cao W., Cerniglia C.E. & Jonson M.G., *The rapid Cyclist*, 12 (1995)

Weiler J., Gausepohl H., Hauser N., Jensen O.N., & Hoheisel J.D., (1997).*Nucleic Acid Res.* **25**, 2792

Incorporation By Reference

All patents, published patent applications and other references disclosed herein are hereby expressly incorporated herein in their entireties by reference.

Equivalents

Those skilled in the art will recognize, or will be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

What is claimed is:

1. A device for integrated processing of a biological sample, said device comprising

a first capillary tube sized for holding the biological sample, said first capillary tube having a first end and a second end,

a second capillary tube sized for holding said biological sample, said second capillary tube having a first end and a second end, and

a connector coupled to the second end of the first capillary tube and the first end of the second capillary tube for mechanically and fluidly connecting the first and second capillary tubes together.

2. The device of claim 1, wherein said connector is a fluid regulating element for selectively providing a fluid seal between the first and second capillary tubes.

3. The device of claim 2, wherein said fluid regulating element comprises a valve or a pinchable silicone connector.

4. The device of claim 1, wherein said second capillary tube is adapted to be removably and replaceably coupled to the first capillary tube by the connector.

5. The device of claim 1, wherein said second capillary tube is adapted to be removably and replaceably coupled to said connector, said connector being adapted to selectively place said first and second capillary tubes in fluid communication with each other.

6. The device of claim 1, further comprising a support assembly for supporting the first capillary tube at said first end.

7. The device of claim 6, wherein said support assembly comprises a fluid handling element fluidly coupled to said first end of said first capillary tube, and a pump for forcing fluid into and out of the fluid handling element.

8. The device of claim 7, further comprising a second connector coupled to the first end of the first capillary tube and disposed between the fluid handling element and the capillary tube.

9. The device of claim 7, wherein said fluid handling element comprises a syringe-type fluid housing.

10. The device of claim 7, wherein said fluid handling element comprises at least a portion of a syringe.

11. The device of claim 8, wherein said second connector comprises a fluid regulating element.

12. The device of claim 1, further comprising a fluid tip coupled to said second end of said second capillary device for introducing fluid to or dispensing fluid from said second capillary tube.

13. The device of claim 1, further comprising a second connector coupled to the second end of the second capillary tube, and a fluid tip coupled to said second connector for forming an entrance or exit aperture in said device for introducing fluid to or dispensing fluid from said second capillary tube, wherein said second connector selectively forms a fluid seal between the fluid tip and the second capillary tube.

14. The device of claim 1, wherein said first capillary tube comprises a glass capillary tube.

15. The device of claim 1, wherein said first capillary tube comprises a silica capillary tube.

16. The device of claim 1, wherein said first capillary tube is coated with a polyimide material.

17. The device of claim 1, wherein said first capillary tube is coated with a polyimide material to facilitate temperature processing of the biological sample when resident within the first capillary tube.

18. The device of claim 1, wherein said connector comprises a valve disposable between an open position for placing said first capillary and said second capillary tube in fluid communication with each other, and a closed position for fluidly sealing said first capillary tube from said second capillary tube.

19. The device of claim 1, wherein said second capillary tube comprises one or more membrane elements for separating components in the biological sample when resident within the second capillary tube.

20. The device of claim 19, wherein said membrane element comprises a semi-permeable microfiber.

21. The device of claim 1, wherein said first and second capillary tubes are sized and configured for holding said biological sample occupying a volume ranging from 10 μl to 0.05 μl.

22. A device for integrated processing of a biological sample, said device comprising

a first capillary tube sized for holding the biological sample, said first capillary tube having a first end and a second end,

a first regulating element coupled to the first end of the first capillary tube for selectively providing a fluid seal thereat,

a second capillary tube sized for holding said biological sample, said second capillary tube having a first end and a second end, and

a second fluid regulating element disposed between the second end of the first capillary tube and the first end of the second capillary tube for selectively providing a fluid seal between the first and second capillary tubes.

23. The device of claim 22, wherein said second capillary tube is adapted to be removably and replaceably coupled to the second fluid regulating element.

24. The device of claim 22, wherein said second capillary tube is adapted to be removably and replaceably coupled to the second fluid regulating element, and said second fluid regulating element is adapted to selectively place said first and second capillary tubes in fluid communication with one another.

25. The device of claim 22, further comprising a support assembly for supporting the first capillary tube at said first end.

26. The device of claim 25, wherein said support assembly comprises

a fluid handling element fluidly coupled to said first end of said first capillary tube, and

a pump for forcing fluid into and out of the fluid handling element.

27. The device of claim 26, wherein said fluid handling element comprises a syringe-type fluid housing.

28. The device of claim 26, wherein said fluid handling element comprises at least a portion of a syringe.

29. The device of claim 22, further comprising a fluid tip coupled to said second end of said second capillary device for introducing fluid to or dispensing fluid from said second capillary tube.

30. The device of claim 22, further comprising

a third fluid regulating element coupled to the second end of the second capillary tube, and

a fluid tip fluidly coupled to said third fluid regulating element and forming an entrance or exit aperture in said device for introducing fluid to or dispensing fluid from said second capillary tube, wherein said third fluid regulating element selectively forms a fluid seal between the fluid tip and the second capillary tube.

31. The device of claim 22, wherein said first capillary tube comprises one of a glass and a silica capillary tube.

32. The device of claim 22, wherein said first capillary tube is coated with a polyimide material.

33. The device of claim 22, wherein said first capillary tube is coated with a polyimide material to facilitate thermal processing of the biological sample when resident within the first capillary tube.

34. The device of claim 22, wherein said first fluid regulating element comprises a valve disposable between an open position for placing said first capillary and said second capillary tube in fluid communication with each other, and a closed position for fluidly sealing said first capillary tube from said second capillary tube.

35. The device of claim 22, wherein said first or second fluid regulating elements comprises a pinchable silicone connector.

36. The device of claim 22, wherein said second capillary tube comprises one or more membrane elements for separating components in the biological sample when resident within the second capillary tube.

37. The device of claim 36, wherein said membrane element comprises a semi-permeable microfiber.

38. The device of claim 22, wherein said first and second capillary tubes are sized and configured for holding microliters of the selected fluid.

39. A device for integrated processing of a biological sample, said device comprising

a first capillary tube sized for holding the biological sample and adapted for thermal processing the sample, said first capillary tube having a first end and a second end,

a second capillary tube sized for holding said biological sample, said second capillary tube having a first end and a second end, and

a fluid regulating element coupled to the second end of the first capillary tube and the first end of the second capillary tube for mechanically and fluidly connecting the first and second capillary tubes together.

40. A modular device for integrated processing of a biological sample, said device comprising

a first modular array of capillary tubes for holding the biological sample, said first modular array having a first array end and a second array end,

a second modular array of capillary tubes for holding the biological sample, said second modular array coupled to said second array end of said first modular array, wherein said second modular array is removably and replaceably coupled to said first modular array, and

an array of fluid regulating elements coupled to the second array end of the first modular array and a first end of said second modular array for selectively providing a fluid seal between the first and second modular arrays of capillary tubes.

41. The modular device of claim 40, further comprising a support assembly coupled to the first array end of the first modular array for supporting the array at said first end,

42. A system for automatic and integrated processing of a biological sample, said system comprising

a first capillary tube for holding the biological sample, said first capillary tube having a first end and a second end,

a support assembly for supporting the capillary tube at said first end,

a second capillary tube for holding said biological sample, said second capillary tube having a first end and a second end,

a first fluid regulating element disposed between the second end of the first capillary tube and the first end of the second capillary tube for selectively providing a fluid seal between the first and second capillary tubes, and

a plate handling assembly positioned for selectively disposing a fluid reservoir beneath the second capillary tube during operation.

43. The system of claim 42, further comprising a second fluid regulating element disposed between the support element and the first end of the first capillary tube for selectively providing a fluid seal between the support element and the first capillary tube,

44. A system for automatic and integrated processing of a biological sample, said system comprising

a first capillary tube for holding the biological sample, said first capillary tube having a first end and a second end,

a support assembly for supporting the capillary tube at said first end,

a first fluid regulating element disposed between the support element and the first end of the first capillary tube for selectively providing a fluid seal between the support element and the first capillary tube,

a second capillary tube for holding said biological sample, said second capillary tube having a first end and a second end,

a second fluid regulating element disposed between the second end of the first capillary tube and the first end of the second capillary tube for selectively providing a fluid seal between the first and second capillary tubes, and

a plate handling assembly positioned for selectively disposing a fluid reservoir beneath the second capillary tube during operation.

45. The system of claim 44, wherein said plate handling assembly comprises a support surface for supporting a fluid reservoir, and means for moving the fluid reservoir into the selected position.

46. The system of claim 44, further comprising a controller in communication with one of said fluid regulating elements for automatically positioning said regulating element between an open position for placing said first capillary and said second capillary tube in fluid communication with each other, and a closed position for fluidly sealing said first capillary tube from said second capillary tube.

47. The system of claim 45, wherein said second capillary tube is adapted to be removably and replaceably coupled to the second fluid regulating element, and said second fluid regulating element is adapted to selectively place said first and second capillary tubes in fluid communication with each other.

48. The system of claim 44, further comprising

a third fluid regulating element coupled to the second end of the second capillary tube, and

a fluid tip coupled to said third fluid regulating element and forming an entrance or exit aperture in said device for introducing fluid to or dispensing fluid from said second capillary tube, wherein said third fluid regulating element selectively forms a fluid seal between the fluid tip and the second capillary tube.

49. The system of claim 44, wherein said support assembly comprises a fluid handling element fluidly coupled to said first end of said first capillary tube, and a pump for forcing fluid into and out of the fluid handling element.

50. The system of claim 44, wherein said first capillary tube comprises one of a glass and a silica capillary tube.

51. The system of claim 44, wherein said first capillary tube is coated with a polyimide material.

52. The system of claim 44, wherein said first capillary tube is coated with a polyimide material to facilitate temperature processing of the biological sample when resident within the first capillary tube.

53. The system of claim 44, wherein said first fluid regulating element comprises a valve disposable between an open position for placing said first capillary and said second capillary tube in fluid communication with each other, and a closed position for fluidly sealing said first capillary tube from said second capillary tube.

54. The system of claim 44, wherein said first or second fluid regulating elements comprise a pinchable silicone connector.

55. The system of claim 44, wherein said second capillary tube comprises one or more membrane elements for separating components in the biological sample when resident within the second capillary tube.

56. The system of claim 55, wherein said membrane element comprises a semi-permeable microfiber.

57. The system of claim 44, wherein said first and second capillary tubes are sized and configured for holding microliters of the selected fluid.

58. A modular system for automatically processing a biological sample, said system comprising

a first modular array of capillary tubes for holding the biological sample, said first modular array having a first array end and a second array end,

a support assembly coupled to the first array end of the first modular array for supporting the array at said first end,

a second modular array of capillary tubes for holding the biological sample, said second modular array coupled to said second array end of said first modular array, wherein said second modular array is removably and replaceably coupled to said first modular array, and

a plate handling assembly positioned for selectively disposing a fluid reservoir beneath the second modular array of capillary tubes during operation.

59. The modular system of claim 58, further comprising a first array of fluid regulating elements coupled to the second array end of the first modular array and the first end of said second modular array for selectively providing a fluid seal between the first and second modular arrays of capillary tubes.

60. The modular system of 59, further comprising a second array of fluid regulating elements coupled to the first array end of the first modular array and the support assembly for selectively providing a fluid seal between the support element and the first capillary tube.

61. The device of claim 1, wherein said first capillary tube is adapted for temperature processing of said sample.

62. The device of claim 1, wherein said first capillary tube is adapted for thermocycling of said sample.

63. The device of claim 1, wherein said second capillary tube comprises separation means for purifying said sample by molecular size discrimination.

64. The device of claim 63, wherein said separation means comprises a semi-permeable microfiber for dialysis of said sample.

65. The device of claim 1 further comprising means for passing a fluid over said first capillary tube.

66. The device of claim 1, wherein said first capillary tube is adapted for thermocycling of said sample, and said second capillary tube comprises separation means for dialysis of said sample.

67. The device of claim 66, wherein said biological sample comprises a polynucleotide, polypeptide, carbohydrate, or mixtures thereof.

68. The device of claim 67, wherein said polynucleotide comprises DNA.

69. A device for dialysis of a biological microsample, said device comprising a capillary tube sized for holding said microsample and comprising separation means for purifying said microsample by molecular size discrimination.

70. A modular device for dialysis of a biological microsample, said device comprising a modular array of capillary tubes sized for holding said microsample, each of said tubes comprising separation means for purifying said microsample by molecular size discrimination.

71. A device for integrated processing of a biological microsample, said device comprising

a first capillary tube sized for holding the biological microsample and adapted for thermocycling the sample, said first capillary tube having a first end and a second end,

a second capillary tube sized for holding said biological sample and adapted for dialysis of the sample, said second capillary tube having a first end and a second end, and

a connector coupled to the second end of the first capillary tube and the first end of the second capillary tube for mechanically and fluidly connecting the first and second capillary tubes together.

72. The device of claim 71, wherein said second capillary tube comprises separation means for purifying the sample by molecular size discrimination.

73. The device of claim 72, wherein said separation means comprises a semi-permeable microfiber.

74. A modular device for integrated processing of a biological microsample, said device comprising

a first modular array of capillary tubes sized for holding the biological microsample and adapted for thermocycling said sample, said first modular array having a first array end and a second array end,

a second modular array of capillary tubes sized for holding the biological microsample and adapted for dialysis of said sample, said second modular array having a first array end and a second array end, wherein said second modular array is coupled to said second array end of said first modular array, and wherein said second modular array is removably and replaceably coupled to said first modular array, and

an array of connectors coupled to the second array end of the first modular array and the first end of said second modular array for selectively providing a fluid seal between the first and second modular arrays of capillary tubes.

75. A method for processing a biological sample, said method comprising the steps of

providing a first capillary tube sized for holding the biological sample,

providing a second capillary tube sized for holding said biological sample,

mechanically and fluidly coupling a second end of the first capillary tube and a first end of the second capillary tube together,

supporting the first capillary tube at a first end, and

positioning a fluid reservoir beneath the second capillary tube during operation.

76. A method for integrated processing of a biological microsample comprising

temperature treating said microsample, and

purifying said microsample,

wherein said temperature treating and purifying steps are carried out in a device that integrates said steps, such that integrated processing of said microsample is achieved.

77. The method of claim 76, wherein said device is a device according to claim 71.

78. The method of claim 76 wherein said device is a device according to claim 74.

79. The method of claim 76, wherein said temperature treating step comprises thermocycling and said purifying step comprises dialysis.

80. The method of claim 79, wherein said microsample comprises a polynucleotide, polypeptide, carbohydrate, or mixtures thereof.

81. The method of claim 80, wherein said polynucleotide comprises DNA.

82. The method of claim 81, wherein said microsample is dialyzed to removed unwanted components of a reaction selected from the group consisting of polymerase chain reactions, DNA sequencing reactions, oligonucleotide extension reactions, exonuclease reactions, OLA reactions, hybridization reactions, and allele-specific polymerase chain reactions.

83. The method of claim 80, wherein said microsample occupies a volume ranging from 10 μl to 0.05 μl

84. A method for conducting dialysis on a biological microsample comprising introducing said microsample into a capillary tube which comprises separation means for purifying the sample by molecular size discrimination, and allowing said microsample to reside in said capillary tube for a time sufficient such that dialysis of said sample is achieved.

85. The method of claim 84, wherein said dialysis is conducted to remove undesired components of a reaction selected from the group consisting of polymerase chain reactions, DNA sequencing reactions, oligonucleotide extension reactions, exonuclease reactions, OLA reactions, hybridization reactions, and allele-specific polymerase chain reactions.

86. The method of claim 84, wherein said microsample comprises a polynucleotide, polypeptide, carbohydrate, or mixtures thereof.

87. The method of claim 86, wherein said polynucleotide comprises DNA.

88. The method of claim 84, wherein said microsample occupies a volume ranging from 10 μl to 0.05 μl.

89. The method of claim 84, wherein said separation means comprises one or more membrane elements.

90. The method of claim 89, wherein said membrane element comprises a semi-permeable microfiber.

91. The method of claim 90, wherein said microfiber is a hollow fiber dialysis tube.

5

92. The method of claim 91, wherein said dialysis tube has a molecular weight cut-off about 100 Kdal.

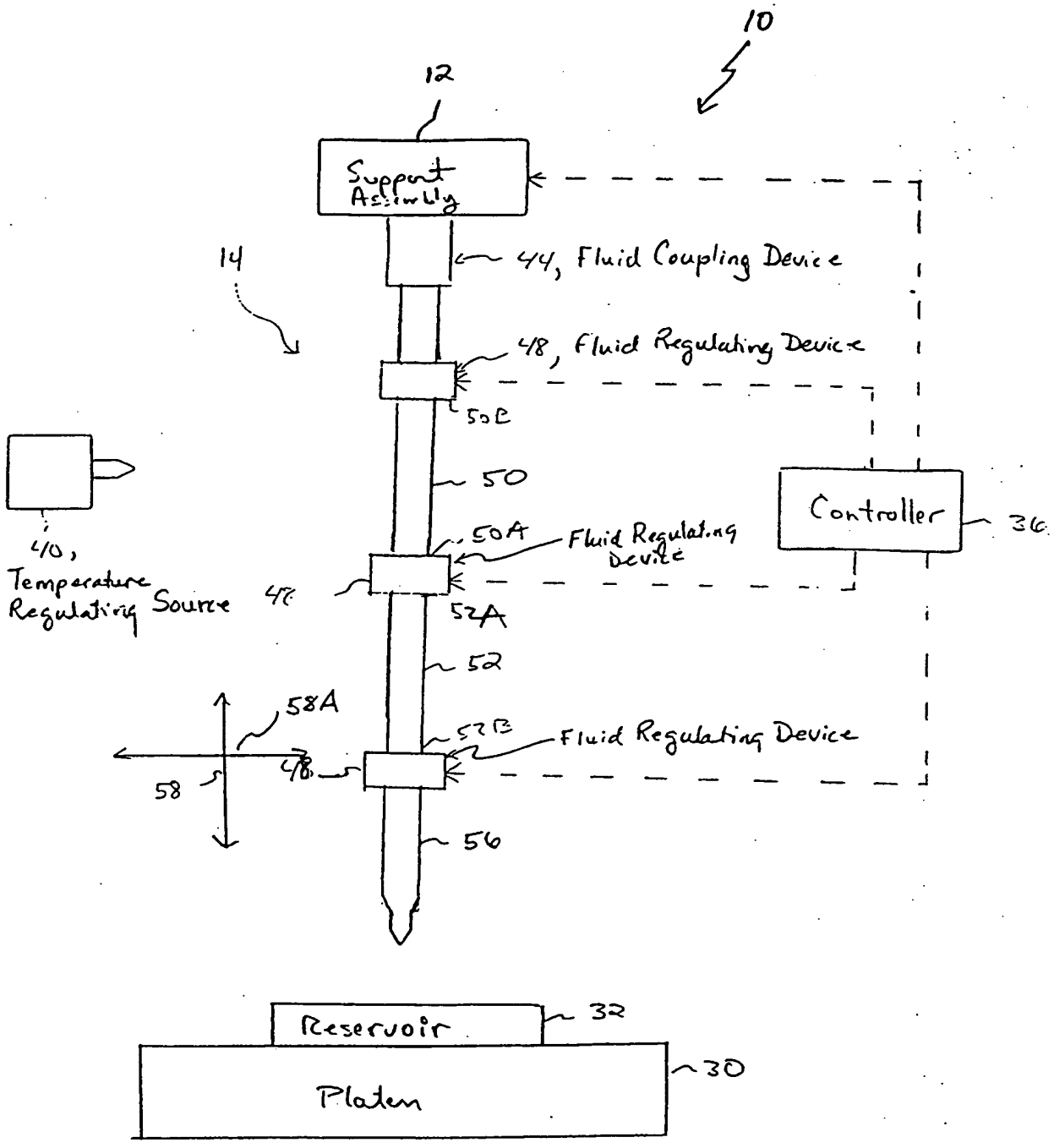

10
12
Support Assembly
14
44, Fluid Coupling Device
48, Fluid Regulating Device
50B
50
50A
Fluid Regulating Device
Controller
36
40, Temperature Regulating Source
46
52A
52
58A
52B
Fluid Regulating Device
58
48
56
Reservoir
32
Platen
30

FIG. 1

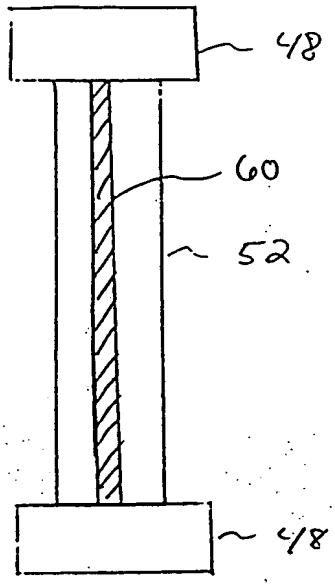


FIG. 2

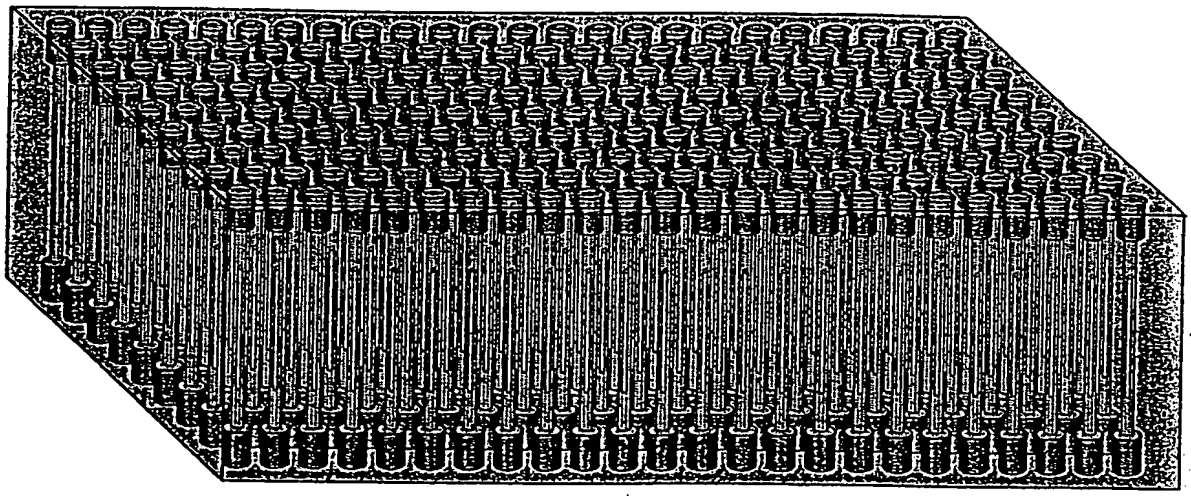


FIG. 3

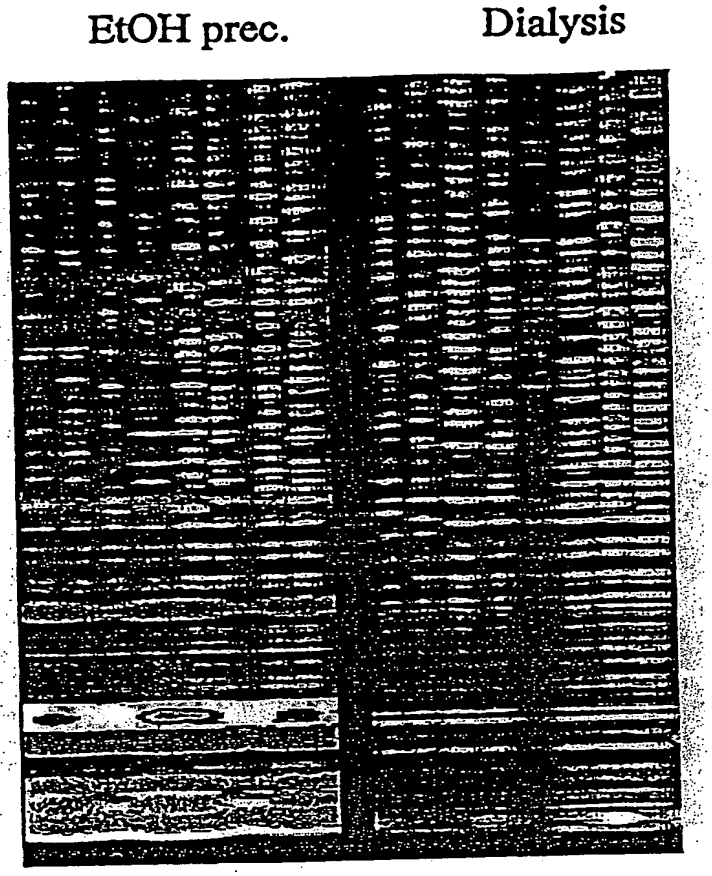


Fig. 4

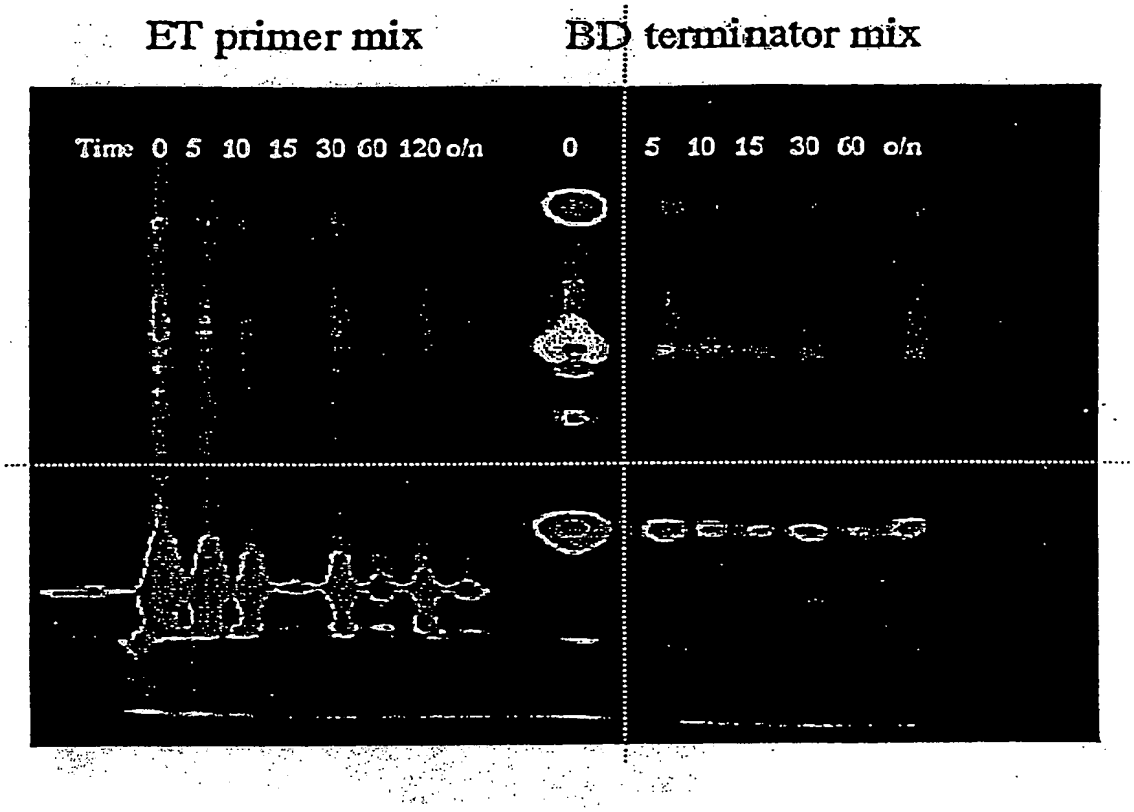

ET primer mix
BD terminator mix
Time 0 5 10 15 30 60 120 o/n
0
5 10 15 30 60 o/n

Fig. 5

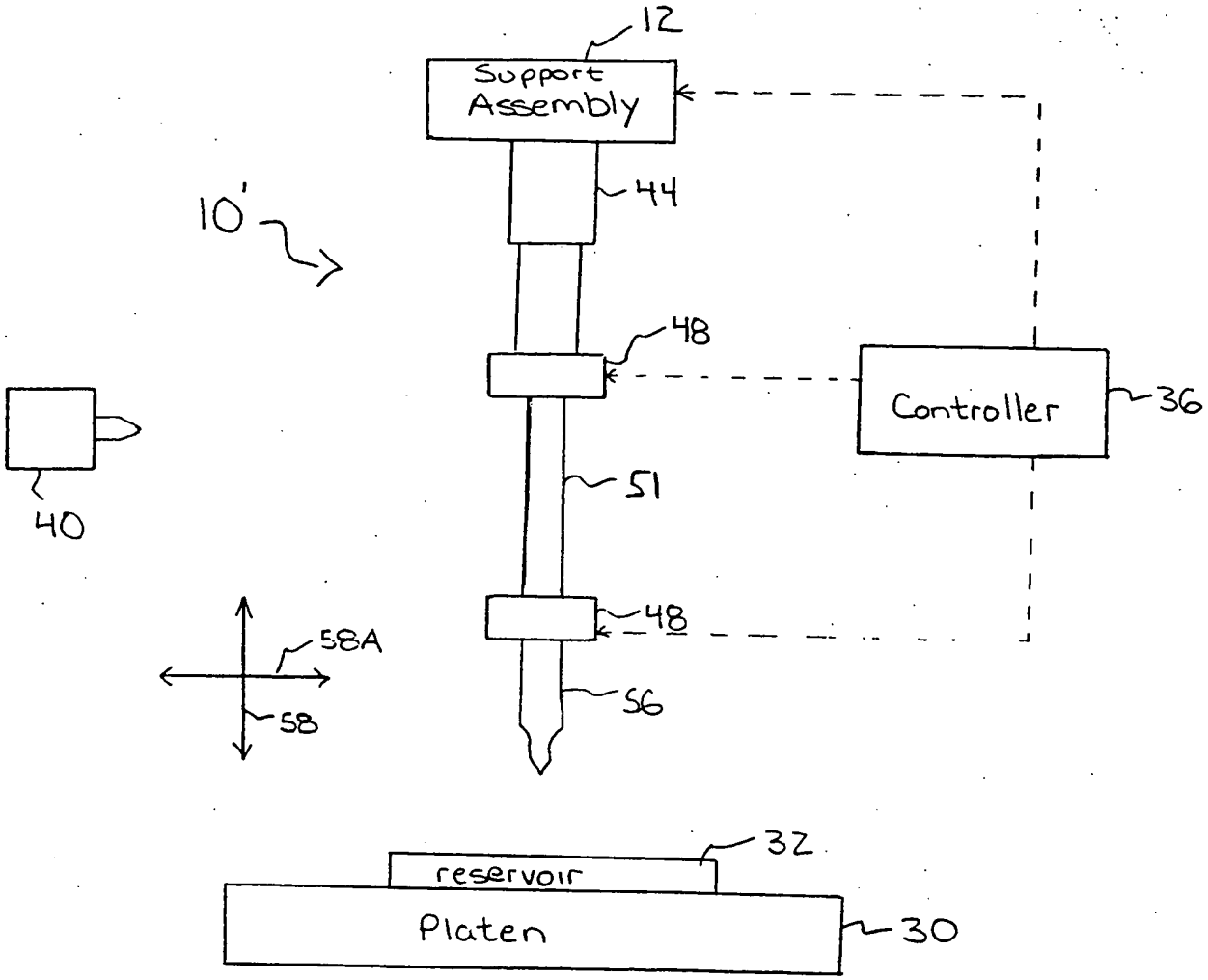

12
Support Assembly
10'
44
48
Controller
36
40
51
48
58A
58
56
32
reservoir
Platen
30

FIG. 6

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
9 November 2000 (09.11.2000)

PCT

(10) International Publication Number
WO 00/66995 A3

(51) International Patent Classification[7]: B01L 3/00, C12Q 1/68, B01J 19/00, B01L 7/00, 3/02

(21) International Application Number: PCT/US00/11371

(22) International Filing Date: 29 April 2000 (29.04.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/131,660 29 April 1999 (29.04.1999) US
60/155,299 21 September 1999 (21.09.1999) US

(71) Applicant: GENOME THERAPEUTICS CORPORATION [US/US]; 100 Beaver Street, Waltham, MA 02453 (US).

(72) Inventors: CAHILL, Patrick; 42 Elliot Street, Natick, MA 02160 (US). SMITH, Douglas; 2 Mayflower Lane, Gloucester, MA 01930 (US). THOMANN, Ulrich; 110 Shade Street, Lexington, MA 02421 (US). ENGELSTEIN, Marcy; 48 Fuller Brook Avenue, Needham, MA 02192 (US).

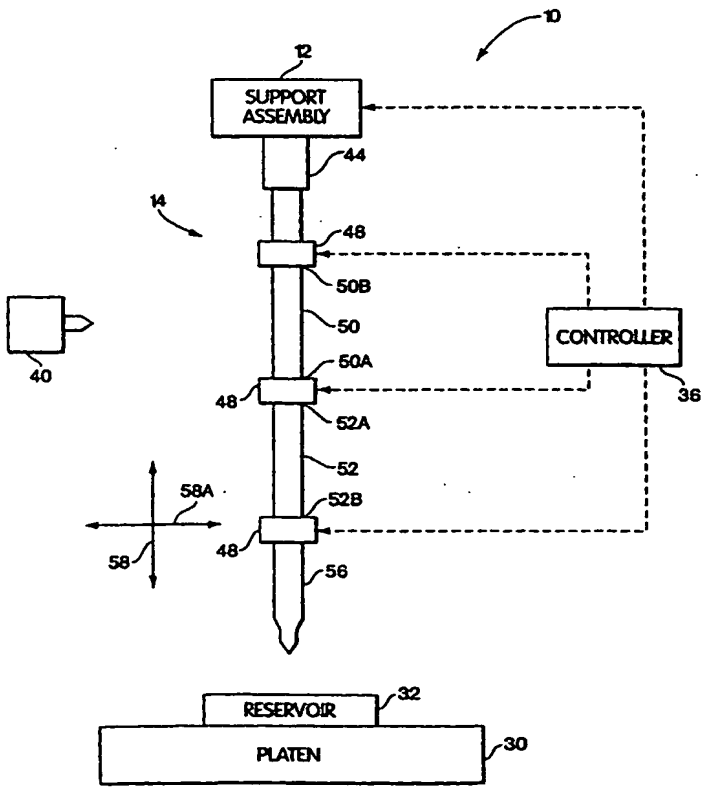
(74) Agents: LAURO, Peter, C. et al.; Lahive & Cockfield, LLP, 28 State Street, Boston, MA 02109 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent

[Continued on next page]

(54) Title: DEVICE FOR RAPID DNA SAMPLE PROCESSING WITH INTEGRATED LIQUID HANDLING, THERMOCYCLING, AND PURIFICATION



(57) Abstract: The present invention provides an automated device to facilitate rapid processing of sub-microliter samples using chemical or enzymatic reactions that involve liquid handling, one or more controlled temperature incubation steps (including temperature cycling), and a purification step based on molecular size discrimination. Exemplary applications include but are not limited to polymerase chain reaction (PCR), DNA sequencing applications, oligonucleotide ligation, ligase chain reaction (LCR), single nucleotide extension, exonuclease treatment, and oligonucleotide hybridization assays.

WO 00/66995 A3

(AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
26 July 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

Interr nal Application No
PCT/US 00/11371

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01L3/00 C12Q1/68 B01J19/00 B01L7/00 B01L3/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B01L C12Q G01N B01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, IBM-TDB

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 769 216 A (CHANDLER HOWARD M ET AL) 6 September 1988 (1988-09-06) column 2, line 5 - line 21 column 7, line 16 -column 8, line 27; figure 2 ---	1,6-10, 12,14, 15,21,75
X	US 5 449 064 A (HOGAN BARRY L ET AL) 12 September 1995 (1995-09-12) column 6, line 65 -column 7, line 63 ---	1-3
X	WO 99 13312 A (MOLECULAR DYNAMICS INC) 18 March 1999 (1999-03-18) page 1, line 29 -page 2, line 3 page 2, line 32 - line 36 page 3, line 9 - line 20 figures 11-13; examples 1-3 figure 15; example 4 --- -/--	1-3

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 October 2000	12.01.01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	HOCQUET, A

Form PCT.ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/11371

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97 48818 A (UNIV LELAND STANFORD JUNIOR) 24 December 1997 (1997-12-24) page 7, line 25 -page 8, line 12 page 9, line 15 -page 10, line 11 page 11, line 9 - line 14 page 13, line 31 - line 33	1,40
A	EP 0 903 176 A (SQUIBB BRISTOL MYERS CO) 24 March 1999 (1999-03-24) paragraph [0017]; figures paragraph [0077] paragraph [0080] - paragraph [0081]	2,40
A	US 5 856 174 A (FODOR STEPHEN P A ET AL) 5 January 1999 (1999-01-05) column 29, line 64 -column 30, line 14	2
A	US 5 030 555 A (CLEMMONS ROGER M) 9 July 1991 (1991-07-09) column 5, line 5 - line 61; figures	1
A	EP 0 805 350 A (UNI DEGLI STUDI DI MILANO) 5 November 1997 (1997-11-05) page 3, line 43 - line 55 page 4, line 39 -page 5, line 18; claim 6 page 5, line 38 - line 43	1,19,55, 56,74
A	EP 0 616 218 A (HITACHI LTD) 21 September 1994 (1994-09-21) column 4, line 16 - line 47; figure 1 column 10, line 16 - line 35; figure 8	1,22,39, 40,42, 44,58, 74,75

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/11371

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-21,22-38,39,40-41,42-43,44-57,58-60,74,75

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-21,22-38,39,40-41,42-43,44-57,58-60,74,75

   devices and methods for processing a sample comprising capillary tubes connected by a fluid regulating element for selectively providing a fluid seal between the capillary tubes

2. Claims: 63, 64,69-73,84-92

   device ans methods for dialysis

3. Claims: 61,62,65-68,76-83

   method for processing a microsample comprising temperature treating and purifying said microsample

INTERNATIONAL SEARCH REPORT

ormation on patent family members

International Application No
PCT/US 00/11371

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4769216 A	06-09-1988	AT 27179 T	15-05-1987
		AT 41528 T	15-04-1989
		AU 553422 B	17-07-1986
		AU 7867881 A	01-07-1982
		WO 8202211 A	08-07-1982
		CA 1184847 A	02-04-1985
		CA 1191786 A	13-08-1985
		DE 3176186 D	19-06-1987
		DE 3177011 D	20-04-1989
		DK 2590 A,B	05-01-1990
		DK 375782 A,B,	20-08-1982
		EP 0067182 A	22-12-1982
		EP 0134605 A	20-03-1985
		JP 57502041 T	18-11-1982
		NO 822750 A	12-08-1982
		NO 904856 A	12-08-1982
		NZ 199286 A	09-05-1986
		NZ 212930 A	20-02-1987
		US 4590157 A	20-05-1986
US 5449064 A	12-09-1995	NONE	
WO 9913312 A	18-03-1999	EP 1019694 A	19-07-2000
WO 9748818 A	24-12-1997	EP 0927265 A	07-07-1999
		US 6132996 A	17-10-2000
		US 5985651 A	16-11-1999
EP 0903176 A	24-03-1999	US 5961925 A	05-10-1999
		CA 2246088 A	22-03-1999
US 5856174 A	05-01-1999	AU 6404996 A	05-02-1997
		EP 0843734 A	27-05-1998
		JP 11509094 T	17-08-1999
		WO 9702357 A	23-01-1997
		US 6043080 A	28-03-2000
		US 5922591 A	13-07-1999
US 5030555 A	09-07-1991	AU 4300089 A	02-04-1990
		WO 9002950 A	22-03-1990
EP 0805350 A	05-11-1997	GB 2312750 A	05-11-1997
		AT 194427 T	15-07-2000
		DE 69702423 D	10-08-2000
		DE 69702423 T	09-11-2000
		US 5976465 A	02-11-1999
EP 0616218 A	21-09-1994	JP 6265447 A	22-09-1994
		US 5480614 A	02-01-1996

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
9 November 2000 (09.11.2000)

PCT

(10) International Publication Number
WO 00/66995 A3

(51) International Patent Classification[7]: B01L 3/00, C12Q 1/68, B01J 19/00, B01L 7/00, 3/02

(21) International Application Number: PCT/US00/11371

(22) International Filing Date: 29 April 2000 (29.04.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/131,660 29 April 1999 (29.04.1999) US
60/155,299 21 September 1999 (21.09.1999) US

(71) Applicant: GENOME THERAPEUTICS CORPORATION [US/US]; 100 Beaver Street, Waltham, MA 02453 (US).

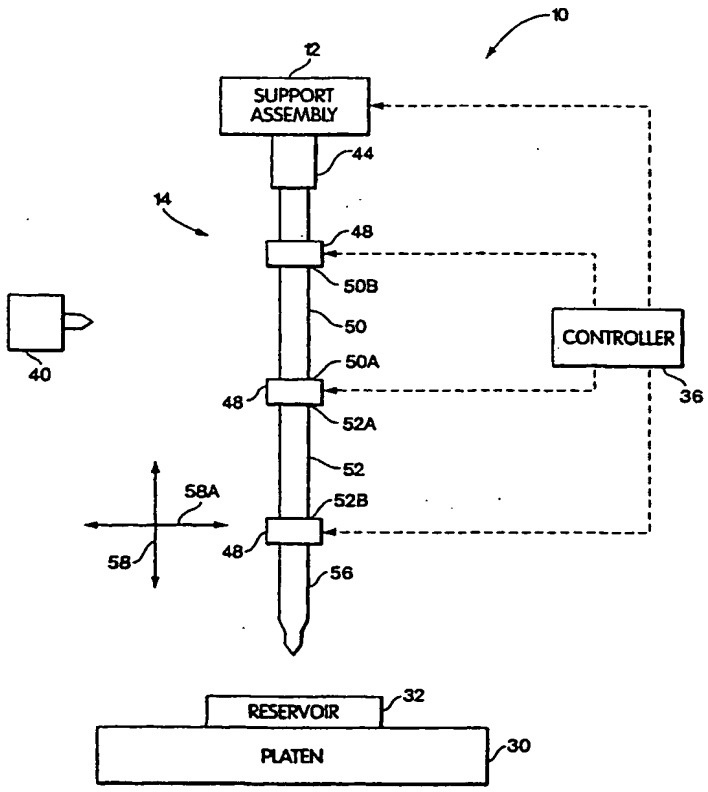
(72) Inventors: CAHILL, Patrick; 42 Elliot Street, Natick, MA 02160 (US). SMITH, Douglas; 2 Mayflower Lane, Gloucester, MA 01930 (US). THOMANN, Ulrich; 110 Shade Street, Lexington, MA 02421 (US). ENGELSTEIN, Marcy; 48 Fuller Brook Avenue, Needham, MA 02192 (US).

(74) Agents: LAURO, Peter, C. et al.; Lahive & Cockfield, LLP, 28 State Street, Boston, MA 02109 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

[Continued on next page]

(54) Title: DEVICE FOR RAPID DNA SAMPLE PROCESSING WITH INTEGRATED LIQUID HANDLING, THERMOCYCLING, AND PURIFICATION



(57) Abstract: The present invention provides an automated device to facilitate rapid processing of sub-microliter samples using chemical or enzymatic reactions that involve liquid handling, one or more controlled temperature incubation steps (including temperature cycling), and a purification step based on molecular size discrimination. Exemplary applications include but are not limited to polymerase chain reaction (PCR), DNA sequencing applications, oligonucleotide ligation, ligase chain reaction (LCR), single nucleotide extension, exonuclease treatment, and oligonucleotide hybridization assays.

WO 00/66995 A3

(84) **Designated States** *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) **Date of publication of the international search report:**
26 July 2001

(48) **Date of publication of this corrected version:**
23 August 2001

(15) **Information about Correction:**
see PCT Gazette No. 34/2001 of 23 August 2001, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

DEVICE FOR RAPID DNA SAMPLE PROCESSING WITH INTEGRATED LIQUID HANDLING, THERMOCYCLING, AND PURIFICATION

Field of the Invention

The invention relates to devices and methods for high speed, low volume automated sample handling of biological samples, which are useful in the field of genomics for a variety of processes, including DNA sequencing, genetic analysis, and gene expression analysis. The invention further relates to devices and methods for setting up and executing assays for high throughput compound screening for pharmaceutical applications.

Background of the Invention

Laboratory automation has played a key role in the advancement of genomics and drug discovery over the past decade. Early work in genomics focused on the automation of fingerprinting and STS mapping procedures through the adaptation of pipetting robots and image acquisition systems (Garcia *et al.*, 1995, Kwok *et al.*, 1992, Lamerdin and Carrano, 1993, MacMurray *et al.*, 1991, Nizetic *et al.*, 1994, Sloan *et al.*, 1993). An interesting example was the "Genomatron" for STS/EST mapping, jointly developed by Intelligent Automation Systems and the Whitehead Institute Genome Center in Cambridge, MA (Dietrich *et al.*, 1995, Hudson *et al.*, 1995). This system performed all the necessary steps for high-speed PCR setup, thermocycling, sample processing for transfer of the reaction products onto nylon membranes, and hybridization with biotinylated probes for CCD based optical signal detection. However, the machine was large, expensive to operate, and could not be easily adapted to performing other tasks. From 1990 onward, a large number and variety of laboratory automation devices became available from an ever expanding set of instrumentation companies. Automated systems are now used in high-throughput sample preparation for DNA sequencing at many of the large sequencing centers.

The degree of automation of the various functions performed in sequencing centers varies widely, ranging from manually fed systems to fully integrated processes. Each extreme has demonstrated positive attributes that contribute to its successful implementation in the sequencing laboratory. However, with a manual setup, there are

significant problems with human error resulting in misidentification of sequencing reads, while the fully integrated process is prone to system failure should one of the modules break.

The current approach in the automation community is to move away from large fully integrated systems to smaller workstations that fulfill specific independent functions in the sequencing process. This means that malfunction of one workstation does not result in a total system breakdown. In addition, this paradigm allows flexibility, which can accommodate changes in requirements as sequencing processes change and improve over time. Specifically, as it takes time to build an automation unit, having flexibility allows one to alter and modify different components as improvements become available. In high throughput sequencing facilities there are several functions which are tedious, inefficient and error prone. Examples of these are colony picking, template preparation, sequencing reaction setup, clone retrieval and gel loading.

Modern laboratories employ partially automated procedures for handling samples. In these procedures, reagents and templates are combined by manually feeding 96-channel pipettors with thermocycling plates. Other laboratories utilize pipetting robots, such as the Tecan Genesis (Ahmadi, 1997) to accomplish the same task. Integrated systems that utilize a variety of pipetting robots, and plate-to-plate liquid transfers, plate sealing, and plate-based thermocycling with magnetic bead or filtration based purification procedures have been constructed. However, these systems are complicated, expensive to build, and suffer from sample evaporation problems and volume constraints.

Another significant drawback in standard 5-10 µl sequencing reactions is that at least 50% of the sample is wasted, never being loaded on the gel. Furthermore, the amount of fluorescently labeled DNA that can be detected on current sequencing machines is much lower than the amounts that are typically processed (0.5-1 µl samples are sufficient).

Several groups have proposed using glass capillaries to handle large numbers of DNA sequencing samples. For example, the first protocols for chemical sequencing developed by Maxam and Gilbert (1977) utilized sealed glass capillaries to handle the samples. In one case, the capillaries are filled, mixed and handled individually as they are moved through several functional "stations" on a conveyor belt type of arrangement

(Friedman and Meldrum, 1998). In another developmental project, 96 capillaries are attached to a Hydra dispenser (Robbins Scientific) so that the samples can be moved up and down past heating elements to perform PCR (Hunicke-Smith, 1997). In a revision of this device, copper heating elements were moved up and down with respect to the position of the samples (Stanford Technology Lab, 1998).

The use of capillaries as sample handling vessels for DNA sequencing has been attempted but, until now, there has been no simple, inexpensive method for accomplishing this purification in very small volumes (< 1μl). Many processes and techniques have been developed to accomplish this task, but the existing techniques have many shortcomings. For example, ethanol precipitation is not fully effective in removing salts and nucleotides and is incapable of removing primers. Furthermore, this technique often results in loss of DNA and is difficult to automate. Commercially available kits such as Quantum Prep ™ SEQueaky Kleen 96 (catalog number 732-6260, Bio-Rad Laboratories, Hercules, CA) and Multiscreen 96 (catalog number MAHVS45, Millipore Corp., Bedford, MA) accomplish the same task as ethanol precipitation, but still do not remove primers, and require a subsequent centrifugation or filtration step. Hence, multiple processing steps are employed in an effort to purify a DNA sample. Effective procedures for complete salt and template removal using spin columns and ultrafiltration have also been reported (Ruiz-Martinez *et al.*, 1998; and Salas-Solano *et al.*, 1998), but the materials are costly and the procedures are not readily amenable to automation.

There exists a need in the art for small-volume DNA purification methods, preferably with re-usable components, that can be easily integrated with capillary-based sample handling, and that eliminate the need for centrifugation. There also exists a need for capillary-based clean-up devices and sample handling alternatives to air-driven thermocycling, and for methods for efficiently handling sample amounts that are just sufficient for each separation, in order to achieve significant cost savings. There also exists a need to minimize sample deterioration and cross-contamination in the aforementioned devices and methods.

Summary of the Invention

The invention relates to an integrated, capillary-based sample handling system for capillary-based aspiration, incubation, purification and delivery of a biological sample that is capable of processing many samples in parallel. The invention further provides integration of pipetting, mixing, temperature treatment, and sample purification that is easy to operate and can be re-used many times.

Thus, in one embodiment, the invention is a device for integrated processing of a biological sample. The device comprises a first capillary tube sized for holding the biological sample, and having a first end and a second end; a second capillary tube sized for holding the biological sample, and also having a first end and a second end; and a connector coupled to the second end of the first capillary tube and the first end of the second capillary tube for mechanically and fluidly connecting the first and second capillary tubes together.

In another embodiment, the device comprises a first capillary tube sized for holding the biological sample, and having a first end and a second end; a first regulating element coupled to the first end of the first capillary tube for selectively providing a fluid seal thereat; a second capillary tube sized for holding the biological sample, and also having a first end and a second end; and a second fluid regulating element disposed between the second end of the first capillary tube and the first end of the second capillary tube for selectively providing a fluid seal between the first and second capillary tubes. In yet another embodiment, the device comprises a first capillary tube sized for holding the biological sample and adapted for thermal processing of the sample, the first capillary tube having a first end and a second end; a second capillary tube sized for holding the biological sample, the second capillary tube also having a first end and a second end; and a fluid regulating element coupled to the second end of the first capillary tube and the first end of the second capillary tube for mechanically and fluidly connecting the first and second capillary tubes together.

In still another embodiment, the invention is a device for integrated processing of a biological microsample. The device comprises a first capillary tube sized for holding the biological microsample and adapted for thermocycling the sample, the first capillary tube having a first end and a second end; a second capillary tube sized for holding the biological sample and adapted for equilibrium dialysis of the sample, the second

capillary tube also having a first end and a second end; and a connector coupled to the second end of the first capillary tube and the first end of the second capillary tube for mechanically and fluidly connecting the first and second capillary tubes together.

Another embodiment of the invention is a device for dialysis of a biological microsample. The device comprises a capillary tube sized for holding the microsample and comprising separation means for purifying the microsample by molecular size discrimination. In a preferred embodiment, the invention is a device for equilibrium dialysis of a biological sample. In another preferred embodiment, the invention is a device for flow dialysis of a biological sample. In yet another preferred embodiment, the invention is a device for exchange dialysis of a biological sample.

In another aspect, the invention is a modular device for integrated handling of biological sample. In one embodiment, the modular device which comprises a first modular array of capillary tubes for holding the biological sample, the first modular array having a first array end and a second array end; a second modular array of capillary tubes for holding the biological sample, the second modular array being coupled to the second array end of the first modular array, wherein the second modular array is removably and replaceably coupled to the first modular array; and an array of fluid regulating elements coupled to the second array end of the first modular array and a first end of the second modular array for selectively providing a fluid seal between the first and second modular arrays of capillary tubes.

In another embodiment, the invention is a modular device for integrated processing of a biological microsample. The device comprises a first modular array of capillary tubes sized for holding the biological microsample and adapted for thermocycling the sample, the first modular array having a first array end and a second array end; a second modular array of capillary tubes sized for holding the biological microsample and adapted for dialysis of the sample, the second modular array also having a first array end and a second array end, wherein the second modular array is coupled to the second array end of the first modular array, and wherein the second modular array is removably and replaceably coupled to the first modular array; and an array of connectors coupled to the second array end of the first modular array and the first end of the second modular array for selectively providing a fluid seal between the first and second modular arrays of capillary tubes.

Another embodiment of the invention is a modular device for dialysis of a biological microsample. The device comprises a modular array of capillary tubes sized for holding the microsample, each of the tubes comprising separation means for purifying the microsample by molecular size discrimination.

In still another aspect, the invention is a system for automatic and integrated processing of a biological sample. In one embodiment, the system comprises a first capillary tube for holding the biological sample, the first capillary tube having a first end and a second end; a support assembly for supporting the capillary tube at its first end; a second capillary tube for holding the biological sample, the second capillary tube also having a first end and a second end; a first fluid regulating element disposed between the second end of the first capillary tube and the first end of the second capillary tube for selectively providing a fluid seal between the first and second capillary tubes; and a plate handling assembly positioned for selectively disposing a fluid reservoir beneath the second capillary tube during operation.

In another embodiment, the system comprises a first capillary tube for holding the biological sample, the first capillary tube having a first end and a second end; a support assembly for supporting the capillary tube at its first end; a first fluid regulating element disposed between the support element and the first end of the first capillary tube for selectively providing a fluid seal between the support element and the first capillary tube; a second capillary tube for holding the biological sample, the second capillary tube also having a first end and a second end; a second fluid regulating element disposed between the second end of the first capillary tube and the first end of the second capillary tube for selectively providing a fluid seal between the first and second capillary tubes; and a plate handling assembly positioned for selectively disposing a fluid reservoir beneath the second capillary tube during operation.

Another aspect of the invention is a modular system for automatically processing a biological sample. The system comprises a first modular array of capillary tubes for holding the biological sample, the first modular array having a first array end and a second array end; a support assembly coupled to the first array end of the first modular array for supporting the array at its first end; a second modular array of capillary tubes for holding the biological sample, the second modular array coupled to the second array end of the first modular array, wherein the second modular array is removably and

replaceably coupled to the first modular array; and a plate handling assembly positioned for selectively disposing a fluid reservoir beneath the second modular array of capillary tubes during operation.

In another aspect, the invention is a method for processing a biological sample. In one embodiment, the method comprises providing a first capillary tube sized for holding the biological sample, providing a second capillary tube sized for holding the biological sample; mechanically and fluidly coupling a second end of the first capillary tube and a first end of the second capillary tube together; supporting the first capillary tube at a first end; and positioning a fluid reservoir beneath the second capillary tube during operation.

In another embodiment, the invention is a method for integrated processing of a biological microsample. The method comprises temperature treating the microsample, and purifying the microsample, wherein the temperature treating and purifying steps are carried out in a device hereinabove described that integrates these steps, such that integrated processing of the microsample is achieved.

In yet another embodiment, the invention is a method of purifying a biological microsample. The method comprises introducing the microsample into a capillary tube which comprises separation means for purifying the sample by molecular size discrimination, and allowing the microsample to reside in the capillary tube for a time sufficient such that purification of the microsample is achieved. In one embodiment, the step of purifying the microsample includes dialysis. In another embodiment, the step of purifying the microsample includes flow dialysis. In another embodiment, the step of purifying the microsample includes exchange dialysis.

In a further embodiment, the invention relates to purifying and cleaning methods that remove contaminants quickly and efficiently from a DNA reaction mix. In a particularly preferred embodiment, the DNA reaction mix comprises DNA templates produced in host cells.

In another embodiment of the invention, the capillaries are designed to prevent leakage, *e.g.*, by sealing, but at the same time to allow penetration, *e.g.*, by a syringe. This arrangement allows for the application of positive or negative pressure. For example, this arrangement permits aspiration of samples from microtiter plates into the capillaries, and subsequent dispensing back into plates for further processing.

In a further embodiment of the invention, multiple samples are thermocycled in a single capillary at the same time.

In another embodiment of the invention, DNA and sequencing reagents are metered and mixed in a single capillary.

In yet another embodiment, the invention provides a guide cap located above the seals at the top and bottom of each capillary tube. This arrangement serves to guide a syringe needle into the opening of the capillary.

In preferred embodiments, the capillary tubes are made of glass, fused silica or TEFLON®.

Brief Description of the Drawings

The foregoing and other objects, features and advantages of the invention will be apparent from the following description and apparent from the accompanying drawings, in which like reference characters refer to the same parts throughout the different views. The drawings illustrate principles of the invention and, although not to scale, show relative dimensions.

FIG. 1 is a schematic perspective view of a capillary based sample handling system in accordance with the teachings of the present invention.

FIG. 2 is a schematic perspective view of one capillary stage for achieving purification of the biological sample of the system of FIG. 1, in accordance with the teachings of the present invention.

FIG. 3 is a schematic perspective of a modular array of capillary tubes suitable for employment with the sample handling system of FIG. 1, in accordance with the teachings of the present invention.

FIG. 4 depicts an electrophoretic gel comparing sequencing reaction clean-up results obtained using an embodiment and method of the present invention (Dialysis) to results obtained using ethanol precipitation, as further described in Example I.

FIG. 5 depicts an electrophoretic gel comparing results obtained using an embodiment and method of the present invention with either ET primer mix or Big Dye Terminator mix as sample, as further described in Example II.

FIG. 6 is a schematic perspective view of a capillary based sample handling system employing a single capillary tube according to the teachings of the present invention.

Detailed Description of the Invention

Definitions

Before further description of the invention, certain terms employed in the specification, examples and appended claims are, for convenience, collected here.

The term "biological sample" refers to a sample comprising one or more cellular or extracellular components of a biological organism. Such components include, but are not limited, to nucleotides (*e.g.*, DNA, RNA, fragments thereof and plasmids), peptides (*e.g.*, structural proteins and fragments thereof, enzymes, *etc.*), carbohydrates, *etc.* The biological samples described herein may also include transport media, biological buffers and other reagents well know in the art for carrying out the processes described above. Although the methods of the invention can be carried out with a biological sample of just about any volume, biological samples in accordance with the invention typically have microliter (μL) volumes and therefore can be referred to as microsamples, *e.g.*, biological microsamples. The methods of the invention are advantageously practiced with biological samples having volumes ranging from 10 μl to 0.05 μL, preferably 0.1 μL to 3 μL.

The term "cassette" refers to a structure or "module" capable of accommodating an array of capillary tubes handling a plurality of samples, *e.g.*, 96 or more samples.

The term "dialysis" is art-recognized and is understood to refer to the separation of substances in solution by means of their unequal diffusion through a membrane. As used herein, "equilibrium dialysis" refers to dialysis which occurs without exchange or flow of dialysate. "Flow dialysis" refers to dialysis which occurs with a flow (or

counterflow) of dialysate. "Exchange dialysis" refers to dialysis which includes at least one change of the dialysate surrounding the membrane.

The term "integrated processing" refers to a process comprising at least two distinct steps which are "integrated" in the sense that the steps are carried out in a single operation. Such a process includes, but is not limited to, template purification, polymerase chain reaction (PCR), DNA sequencing, polynucleotide ligation, cloning, ligase chain reaction (LCR), single nucleotide extension reaction, exonuclease treatment, and oligonucleotide hybridization reactions. Process steps associated with these processes include, for example, the aspiration, mixing, incubation, purification, temperature treating, such as heating or cooling, and delivery of the biological sample alone or in a biologically compatible carrier fluid in a selected manner. The invention described herein integrates these distinct steps; *i.e.*, the invention makes it possible for these steps, heretofore carried out in separate operations, to be carried out in a single operation.

The term "membrane element" refers to a material which may used to separate substances in solution by means of unequal diffusion, *e.g.*, by size exclusion. Exemplary membrane elements are semipermeable; i.e., the membrane elements are capable of permitting dialysis to take place.

The term "purification" is intended to encompass, in its various grammatical forms and synonyms (*e.g.*, purification, purifying, clean up, *etc.*) any operation whereby an undesired component(s) is/are separated from a desired component(s). Such operations include, but are not limited to, filtration, ultrafiltration, dialysis/equilibrium dialysis, chromatography, *etc.* In certain embodiments, purification is achieved by molecular size discrimination among the components of the biological sample. Purification by molecular size discrimination can be achieved using any number of materials of varying porosity well known in the art including, but not limited to, filters, membranes, and semipermeable ultrafiltration fiber materials.

The terms "temperature processing," "temperature treating," and "thermal processing" are used interchangeably herein to refer to the application of a variety of temperature conditions to the sample, depending on the particular process underway and include, but are not limited to, continuous and discontinuous heating regimens, *e.g.*, denaturation, annealing, incubation, precipitation, *etc.* For example, the terms broadly

encompass thermocycling associated with PCR and similar processes. The term "ultrafiltration" refers to any method of dialysis wherein the sample is under positive pressure relative to the dialysate.

The invention described herein includes an integrated capillary-based sample handling system for automated capillary-based processing of the present invention. The system is capable of processing many samples in parallel, if desired, using standard micro-titer plates as reagent sources. The use of capillary tubes in connection with the present invention has the advantage that only a small fraction of the liquid volume is exposed to the atmosphere, so that evaporation is minimized. This promotes the processing of the sample, while concomitantly eliminating or reducing sample loss. The capillary tubes of the system can be used to retrieve, mix and dispense fluids by integration with air or liquid-filled volumetric devices, such as piezoelectric elements, movable pistons or syringe-type plungers.

Figure 1 is a schematic depiction of the capillary based sample handling system 10 in accordance with the teachings of the present invention. The illustrated system 10 employs a support assembly 12 that is configured for supporting and/or mounting a capillary based processing assembly 14. The support assembly 12 positions the capillary based processing assembly 14 over a platen surface 30 that is sized and dimensioned for supporting a reservoir plate 32. A controller 36 is coupled to selected components of the sample handling system 10, such as the support assembly and various portions of the processing assembly 14, as well as to a temperature regulating source 40. The support assembly 12, platen 30, and control 36 can form part of a conventional fluid dispensing unit, such as a Hydra dispenser, manufactured by Robbins Scientific, U.S.A., and which can be modified in an appropriate manner obvious to the ordinarily skilled artisan in light of the teachings herein to operatively mount the capillary based processing assembly 14. Those of ordinary skill will also recognize that other fluid dispensing and sample handling units presently employed and in practice, whether in modular or discrete forms, can be employed to perform the features and functions described herein, provided they are adapted to mount the capillary based processing assembly 14 of the present invention.

The illustrated processing assembly 14 includes multiple, discrete components that are functionally and operatively connected in a selected manner so as to perform multiple processing steps upon a biological sample. The capillary based processing assembly 10 includes multiple, operatively connected capillary tubes that are coupled in a selected fashion to perform multiple processing steps in a single, vertically integrated unit. The illustrated assembly 14 includes a fluid coupling 44 that is connected at one end to the support assembly 12, and at an opposite end to a fluid regulating device 48. The fluid coupling 44 operates in connection with the controller 36 and support assembly 12 to regulate fluid movement within the processing assembly 14. For example, the fluid coupling 44 is coupled to a pump mechanism, which forms part of the support assembly 12, to either introduce fluid into or discharge fluid from a proximal end of the processing assembly 14. Those of ordinary skill in the art will readily recognize that the fluid coupling 44 can comprise any suitable self contained fluid actuating mechanism, such as a plunger-type or a syringe-type mechanical coupling, or can be any other suitable fluid conduit that serves as a fluid and mechanical coupling between the support assembly 12, which can comprise a fluid movement or pump mechanism, and the remainder of the processing assembly 14. The fluid coupling can also optionally include a guide for a syringe/syringe needle at the top and/or bottom of the capillary tube 50.

With further reference to Figure 1, the illustrated fluid regulating device 48 can be a valve-type structure, such as a fluid valve or pinchable silicone connector, that is adapted for selectively placing the fluid coupling 44 and selected fluid communication with the remainder of the processing system, such as the capillary tube 50. The fluid regulating device 48 can either be operated manually, or can be part of an overall automated sample handling system, such as the automated sample handling system 10 of the present invention, by coupling via any suitable communication pathway to the illustrated controller 36. The capillary tube 50 can be a commercially standard and available capillary element that is sized and dimensioned for fluidly retaining microliters of a biological fluid. According to a preferred practice, the illustrated capillary tube 50 is adapted for holding a biological sample in a biologically compatible carrier medium. Examples of suitable biological samples include DNA, proteins and other like biological

components, and the biologically compatible carrier medium can be any known buffer or processing fluid typically employed in biological processing techniques.

The illustrated capillary tube 50 is further coupled at a proximal end 50A to a second fluid regulating device 48. The fluid regulating device 48 is preferably similar to the fluid regulating device coupled to the distal end 50B of the capillary tube 50. The combination of the pair of fluid regulating devices 48, (48 coupled to either end of the tube 50 can fluidly isolate the chamber of the capillary tube 50 from the remainder of the processing system 14). This configuration allows a selected processing regimen as implemented by the controller or by hand to occur within the confines of the capillary tube independent of any other activities being performed in any other section of the processing assembly 14. For example, a temperature regulating source 40, which can include a heating element or a cooling source, is thermally coupled to the capillary tube 50 when the processing assembly 14 is disposed in a selected position. The temperature regulating source 40 is preferably a heating source that heats the capillary tube 50. In this manner, the biological sample and associated carrier fluid, if desired and contained within the chamber of the capillary tube 50, can undergo a thermocycling process according to a user selected regimen. Those of ordinary skill will readily recognize that the temperature regulating source 40 can comprise a series of resistive heating elements, or can be a heating source that passes heated air across the outer surface of the capillary tube 50.

The illustrated capillary tube 50 can be made of glass, or is coated with a polyimide coated fused silica. Suitable capillary tubes for use in accordance with the teaching of the present invention are within the purview of one of ordinary skill, when considering the particular temperature ranges, capillary size, heating duration, and specific contents and quantities of the biological sample and carrier fluid. The ordinarily skilled artisan in light of all these parameters would be able to determine the appropriate capillary tube size and length. Moreover, the particular temperatures in which to heat the contents of the capillary tube are also within the purview of one of ordinary skill when considering the particular type of processing that is desired to be carried out within the capillary tube, and the type and quantity of the biological sample and carrier fluid.

With reference again to Figure 1, a second capillary tube 52 has a distal end 52A coupled to the intermediate fluid regulating device 48, and has a proximal end 52B coupled to a third fluid regulating device 48. As set forth above, the fluid regulating devices 48, coupled to the proximal and distal ends 52A and 52B of the capillary tube 52, also serve to isolate fluidly the capillary tube 52. Consequently, a second or additional processing regimen can occur within the capillary tube 52 independently, contemporaneously and concurrently of any particular processing regimen that occurs within the other capillary tube 50.

A fluid tip 56 having a central lumen is coupled to the third fluid regulating device 48 in accordance with known techniques. The fluid tip 56 can serve to either transfer fluid to or receive fluid from the reservoir 32 disposed on the platen 30 of the sample handling system 10. The illustrated reservoir 32 can be a standard 96-well micro-titer plate, and hence can be employed in connection with the illustrated system 10 to perform multiple, parallel processing of biological samples. The fluid tip is preferably any suitable mechanical coupling that fluidly connects the processing assembly with an external fluid source, such as the fluid reservoir, according to one mode of operation. The tip is preferably TEFLON® coated, and can be made of plastic or stainless steel. Other types of tips obvious to the ordinarily skilled artisan can also be used.

The illustrated processing assembly 14 provides for a single, stacked, and integrated processing sub-assembly 14 that allows for single or multiple biological samples to be aspirated throughout the axial length of the assembly, and can isolate, in connection with the fluid regulating devices, selected portions of the assembly to perform different processing regiments in parallel.

The illustrated control 36 is programmed to control, according to user selected information, the fluid regulating elements 48 to selectively connect or disconnect either of the capillary tubes 50 and 52 relative to each other. For example, the controller 36 can dispose in an open position the illustrated fluid regulating devices 48 to effectively form a single axial fluid lumen along about the entire axial length of the assembly 14; that is, from the support assembly to the exit port of the fluid tip 56. Alternatively, the controller 36 can actuate one or more of the fluid regulating devices 48 to fluidly separate or isolate one or more regions of the processing assembly from the remaining

regions. The illustrated controller 36 also controls the support assembly 12. According to a preferred practice, the controller provides selected control data that instructs the support assembly 12 to position the processing assembly 14 at selected positions to either retrieve or introduce fluid to and from the reservoir 32. The axial movement imparted by the support assembly 12 to the processing assembly 14 is indicated by the arrow 58. The illustrated controller 36 further provides control signals to the temperature regulating source 40 to selectively actuate the source to provide heating or cooling of a particular region of the processing assembly 14. Although the illustrated temperature regulating source 40 is positioned for regulating the temperature of the first capillary tube 50, those of ordinary skill will readily recognize that the support assembly 12 can position other portions of the processing assembly 14, such as the second capillary tube 52, in a position to be heated or cooled by the source 40. Alternatively, multiple sources 40 can be used. Moreover, the illustrated support assembly 12 and/or controller can move the processing assembly 14 in the horizontal direction, as indicated by arrow 58A.

A significant advantage of the illustrated sample handling system 10 is that it provides for an automated, high speed sample handling system that can process low volumes of a biological sample in an automated format, without the need for centrifugation which typically requires large volumes of sample. Moreover, the illustrated processing assembly of the system 10 preferably employs reusable components, thereby significantly extending the useful life of the major system components. The integrated processing assembly 14 also provides for an efficient, compact and relatively simple processing assembly for performing one or more processing regimens in parallel, and if desired, independently of each other.

A significant advantage of employing multiple capillary tubes in the processing assembly 14 is that the sample volumes provided by each capillary tube allows the processing of significantly smaller sample portions, since relatively small volumes of the overall carrier fluid disposed within the capillary tubes are subject to evaporation. This sample conservation advantage significantly reduces the sample volumes necessary to achieve selected processing of the sample, while concomitantly affording sample outputs that have sequencing ladders with improved signal strength and resolution.

According to a preferred practice, the capillary tubes preferably have internal volumes that accommodate fluid sizes of less than about 1 microliter.

An advantage of employing the novel submicroliter capillary tubes, in tandem, as illustrated in Figure 1, is that it allows the use of minimal amounts of expensive sequencing reagents and relatively small volumes of biological samples in an automated sample handling format. The illustrated processing assembly 14 can be used to perform purification procedures on polymerase chain reaction (PCR) products, preparing sequencing ladders, and injecting the sequencing ladders into appropriate microtiter plates, or aspirating the biological products into particular zones of the processing assembly 14. The particular processing regimens which can be accommodated or performed within particular zones of the processing assembly 14 are described in detail below.

According to one practice, the illustrated processing assembly 14 can be employed to purify a biological sample disposed within one or more regions of the processing assembly. For example, as illustrated in Figure 2, the capillary tube 52, which is coupled to a fluid regulating device 48 at either end, can house a separation element, such as a semipermeable microfiber, for processing a biological sample, such as DNA. According to one technique, the capillary 52 and illustrated microfiber 60 can be employed to perform equilibrium dialysis within the confines of a capillary electrophoretic system. Typically, DNA sequencing products are purified to remove excess salt, nucleotides, primers, and templates from the biological sample. The illustrated microfiber 16 can be employed to perform the filtration process upon the DNA, to exclude the desired products, while concomitantly allowing undesired components to pass therethrough when the processing assembly is exposed to a pressure or vacuum condition at a proximal end. The DNA sample is cycled through the microfiber by the pressure formed within the system, thereby resulting in relatively small components being filtered out of the hollow fibers and hence the sample. The use of a capillary tube with one or more microfibers disposed therein, provides for the ability to perform equilibrium dialysis upon very small volumes of between about 10 to 0.05 microliters.

As illustrated in Figure 3, an additional significant advantage of the present invention is that the capillary tubes 50 and 52 can form part of an array of capillary tubes 70, *e.g.*, a cassette of capillary tubes. The illustrated array of capillary tubes allow the illustrated sample handling system 10 to perform multiple, parallel processes in a vast array of processing assemblies 14. In particular, the system 10 can accommodate the use of multiple arrays of the capillary tubes 50 and 52. For example, the capillary tubes 50 can comprise part of an array, and the capillary tube 52 can also form part of a second array of capillary tubes. The illustrated fluid regulating devices 48 can be interposed between the modular capillary tube arrays, to form a modular and stackable assembly of processing tubes for use in connection with the illustrated system 10. The illustrated controller 36 can provide instruction data to a suitable robotic assembly, such as a pipetting robot, to selectively stack together or remove one or more of the modular arrays of capillary tubes 50 and 52 from the processing assembly 14 of the illustrated system 10. This modular aspect of the illustrated system 10 allows for the removable and replaceable use of modular arrays of capillary tubes, in order to aspirate or introduce biological samples to a standard 96-well microtiter plate. Moreover, the stackable aspect of the modular array of capillary tubes allows multiple selected processes to be conducted within one or more of the capillary tubes. Upon completion of the processing regimen, the system can disconnect one or more of the modular arrays with the pipetting robot for downstream processing or storage.

In accordance with another embodiment of the present invention illustrated in Figure 6, the sample handling system 10' can employ a single capillary tube 51 for processing a biological sample. The sample handling system 10' can be used in applications in which it may be desirable to effect a single processing regimen upon the biological sample. Such processing regimens can include sample purification and thermocycling, as well as any other of the sample processing steps described herein. In the case of sample purification, a membrane element, such as the microfiber 60 illustrated in Figure 2, can be provided within the capillary tube 51.

According to the invention, purification of a sample may be achieved by a variety of methods, including dialysis, filtration, ultrafiltration and chromatography. The invention further provides various configurations to achieve purification, depending on the method of purification selected. For example, when equilibrium dialysis is the

method of purification, the apparatus of the invention provides at least one capillary comprising a membrane element in operative contact with a dialysate, *e.g.*, water. In certain embodiments, the dialysate is contained in a cassette. When exchange dialysis is the method of purification method, the capillary may be inserted successively into at least two cassettes containing a dialysate.

As set forth herein, the present invention includes dialysis techniques, which may be used effectively to "clean up" polymerase chain reaction (PCR) and cycle sequencing reactions. Until now, one of the problems with conventional dialysis techniques has been one of scale. Typically, dialysis is carried out on relatively large sample volumes of at least 1 mL or more. The typical PCR or sequencing reaction, on the other hand, generally utilizes sample volumes of approximately 10 μL or less, significantly smaller than the sample volumes in conventional dialysis techniques.

The present invention addresses this disparity by using a membrane element, such as one or more microfibers inserted within one of the capillary tubes. The microfiber performs the same separation functions as the much larger dialysis operations, but with much smaller sample volumes and without the use of centrifugation. The microfibers can be generated or manufactured by removing one or more hollow fibers from commercially available filtration cartridges. Typical cartridges contain many hundreds of fibers, since the cartridge is solely designed to perform dialysis on large sample volumes, *e.g.*, 1 mL or more. Many types and sizes of hollow fiber filtration cartridges are available through such suppliers as Millipore Corp. Bedford, MA or Spectrum Labs Laguna Hills, CA. Typically these cartridges are used as ultrafiltration devices, where the dialysis membrane acts as a filter, excluding the desired products while allowing the undesired components to pass through when pressure or vacuum is applied to the system. The present invention achieves proper filtration or separation of components from small volumes of a biological sample by employing one fiber for each biological sample. In this way, dialysis on sample volumes of 10 to 0.05 μL volumes is achieved.

According to one mode of operation, the present invention achieves appropriate purification of a sample by first performing a standard *Big Dye Terminator Cycle Sequencing Ready Reaction Kit*, part # 4303154 PE Applied Biosystems Foster City CA, on a reaction sample size of between 0.05-10 μl. The sample volume is drawn up into a

hollow fiber filter which has been cut out of a Spectrum cartridge cat # 132229 Spectrum Labs Laguna Hills, CA using a 10 µl syringe from Hamilton, Reno, Nevada (see FIG. 2). Purification is then achieved according to any of the various methods described herein.

One advantage of the present invention is the integration of pipetting, mixing, temperature control/thermocycling, and sample purification in a simple, single integrated flow-through system 10 (or processing assembly 14) that is easy to operate and can be reused a significant number of times. Sample components are aspirated and mixed on a fluid tip conduit 56, such as a TEFLON® coated tip, TEFLON® tubing, other plastic type tubing, stainless steel, or TEFLON® coated stainless. Thermocycling is achieved, for example, by blowing air of different temperatures over a capillary tube 50 made of glass, or more preferably, polyimide coated fused silica, although a liquid medium could also be used for heat transfer. Capillary ultrafiltration material allows the removal of unwanted reaction components that are small in molecular size compared to the desired DNA products by dialysis against water in an adjacent chamber.

The technique of dialysis, although well established, has heretofore been difficult to perform on small sample volumes without suffering loss of the sample. Semi-permeable microfiber ultrafiltration materials are available in a variety of porosities, which allow small components to freely pass through while larger components are selectively retained. Although these are commonly used for ultrafiltration of proteins, only some of the materials are suitable for capillary-based dialysis. Because all of the reaction components to be removed from PCR and DNA sequencing reactions are much smaller than the desirable products, the process of the present invention is an optimal method to "clean up" these reactions.

According to one embodiment of the present invention, each zone of, or processing regimen performed by, the sample handling device (*e.g.*, mixing, cleaning, thermocycling, and liquid handling) can be isolated from the others by the illustrated valves 48 to prevent 'wandering' of the sample during each step of the procedure. A linear or rectangular array of such devices interfaces conveniently with a microtiter plate 32 disposed on the system platen 30. The dialysis and thermocycling sections of the array are enclosed within separate sealed containers to provide controlled liquid or air flow across the devices.

This arrangement allows sub-microliter quantities of liquids to be aspirated by negative pressure on the system. The samples are specifically moved to various parts of the system by controlled pressure changes. Note that in a capillary of diameter 250 μM, a volume of 0.5 μl occupies a height of approximately 1 cm. A number of commercial devices are available on the market that may be adapted in accordance with the teachings of the present invention for small volume aspiration and delivery utilizing capillary sample holders. These include syringe pumps from several manufacturers, 96- and 384- channel sample liquid dispensers from Robbins Scientific, Inc. (Hydras), "Nanomovers" from Precision Scientific, and several other possible designs. The Hydra systems are convenient because of the ability to simultaneously, accurately, and coherently aspirate and deliver selected volumes from parallel channels. These systems offer ease of integration with physical plate-handling systems and PC-based programming systems through an RS232 port.

For temperature control and thermocycling, a two-temperature air-circulation system with appropriately placed valves may be used to enable a wide range of air temperatures to be quickly attained. For example, the heating source 40 can be employed to heat a sample disposed in the capillary tube 50 and isolated from other sections of the processing assembly 14 by a pair of fluid regulating devices 48.

Thus, in one embodiment, the thermocycler will use a combination of hot and cold air to change sample temperature. Simple air blowers or blowing ambient air and air heated by resistance heaters over the capillaries may be used to change the temperature. The temperature may be measured and controlled by standard PID controllers. The heating rate may be increased as desired by using, for example, superheated air for the first part of the heating cycle, then cooler air to avoid excessive overshoot of the temperature of the capillaries.

The in-line processing assembly 14 mounted to the support 12 of the sample handling system 10 integrates sample aspiration, mixing, thermocycling, dialysis and dispensing in a single device. This achieves definite advantages in terms of sample integrity (no evaporation), simplicity and throughput. Optical sensors may be employed in connection with the illustrated system 10 to detect liquid levels at one or more points in the system 10, and provide open loop or feedback control to the controller 36 to adjust, if necessary, the sample or fluid level volumes within one or more sections of the

processing assembly. The present system 10 provides an accurate volumetric system that is much simpler to operate and maintain than conventional systems. For accurate aspiration of small liquid volumes, it is advantageous to maintain a minimal air gap (water can fill almost the entire system) and to coat the outside surface of the capillaries with a hydrophobic material such as TEFLON® to avoid adherent drops and carry-over. Thus, volumes can be accurately aspirated and dispensed at the tip 56, and moved to precise positions within the system. Different solutions (for instance, DNA and *Big Dye Terminator Cycle Sequencing Ready Reaction Mix* (DT-mix)) can be aspirated in separate "slugs" with a small air gap in between (with minimal cross-contamination). When the samples are dispensed back to the tips, the liquid droplet (provided it is not too large relative to the size of the tip) adheres to the tip causing the samples to mix. The droplet can then be aspirated back into the system. Silica capillaries are available with consistent dimensions to within 2 microns.

The invention also relates to purifying and cleaning methods that remove contaminants quickly and efficiently from a DNA reaction mix. Current sequencing machines use electrophoresis through a gel to separate and detect different lengths of DNA that have been appropriately labeled. To make these machines provide results faster and more accurately, the shapes of the gel separation media have gone from thick gels to a gel captured by thin capillaries. A major drawback is the contaminants in the DNA being sequenced tend to physically plug the capillary and interfere with the accurate detection of the different DNA lengths. One major source of contaminants in the DNA sample is the result of by-products of the thermocycling reaction that generates the DNA sample. Both regular and dye-labeled nucleotides that are not incorporated into the DNA strings during the reaction become contaminants that degrade the DNA sequencer. Additionally, ionic components of the reaction reagents remaining in the reaction (*e.g.*, salts) also degrade the machines.

In one embodiment, the present invention provides for effective removal of contaminants from a thermocycling reaction. Once the reaction mixture is thermocycled, purification may be achieved by placing the mixture into a hollow membrane element, which is in contact with a solution having a lower concentration of ionic components. The difference in osmotic pressure across the membrane forces

contaminants in the product to migrate across the membrane into the aqueous solution, effectively removing them from the product.

In another embodiment, the invention provides an apparatus and method for purifying DNA molecules produced in host cells. As further described in Example IV, the invention provides an apparatus and method for producing template DNA in a host cell, lysing the host cell, purifying and sequencing the template DNA.

Exemplification

EXAMPLE I: DNA Sequencing

With reference to Figure 1, the system 14 is first rinsed with water, and the syringes are then filled with water except for about 5 μL. Next , a 1 cm (0.5 μL) air gap is drawn up, followed by 0.5 μl DNA (50 ng/μL), and 0.5 μL of *Big Dye Terminator Cycle Sequencing Ready Reaction Mix* ("DT")(part number 4303154, PE Applied Biosystems, Foster City, CA). DT and DNA are then ejected to mix. 1 μl of the reaction mixture is aspirated and moved up to the thermocycling zone 50 (maintaining a 1-2 cm air gap), and then thermocycling is performed. The sample is moved down to the dialysis zone 52 and dialyzed for 20 min. Finally, the samples are ejected into a microtiter plate and rinsed with water.

During this procedure, a plate handler below changes plates (from DNA source, to DT mix, to output plate) and moves them up and down (to create air gaps). The pipetting, flow-through purification, and thermocycling are fast, so the throughput is very high; *e.g.*, on the order of 384 samples per hour for a 384 channel system. If gels can be run at a rate of 96 samples every 3h (the maximum proposed speed), 1 sample robot could feed 8 gel systems running at full speed.

The sample handling device may also be constructed as a series of independent modules that can be stacked together, or independently attached temporarily to a liquid handling device to effect the liquid handling steps. An advantage of this approach is that a single liquid handling device, which is the most expensive part of the system to build, could be more optimally utilized to process samples in a large number of dialysis and thermocycling modules. The latter modules can then be handled in a similar fashion

as plates are handled on a typical integrated automation system, such that the independent units would be advantageously sealed automatically when detached from the liquid handling device, and that plate-to-plate transfers would be replaced by capillary-to-capillary transfers (avoiding contact with the atmosphere). An example of a dialysis module is shown in Figure 3.

EXAMPLE II: Dialysis

This example further describes the feasibility of using hollow fiber dialysis on very small volumes as a method for sequencing, and PCR reaction clean-up. Most of the work was accomplished on sequencing reactions prepared using PE Applied Biosystems standard *Big Dye Terminator Cycle Sequencing Ready Reaction Kit*, part # 4303154, following the standard 1/4X BigDye Terminator Hydra Sequencing Reactions protocol. The results were obtained on an ABI 377 automated DNA sequencer (PE Applied Biosystems Foster City, CA) or a Megabace 1000 automated DNA sequencer (Molecular Dynamics Sunnyvale, CA). The raw data was analyzed by Phred software (Brent Ewing, LaDeana Hillier, Michael C. Wendl and Phil Green *Base-Calling of Automated Sequencer Traces Using Phred I. Accuracy Assessment* Genome Research 8, pg 175-185; Brent Ewing, Phil Green *Base-Calling of Automated Sequencer Traces Using Phred II. Error Probabilities* Genome Research 8, pg 186-194).

The individual hollow fiber dialysis tubes were obtained by cutting open hollow fiber filtration cartridges produced by Amicon of Beverly, MA and then removing the fibers. Fibers were stored in sterile distilled water until ready for use. Fibers containing different pore sizes were used in these studies but the bulk of the experiments were carried out using filters with an average pore size of 100Kdal.

Standard 1/4X BigDye Terminator Hydra Sequencing Reactions were transferred to the hollow fiber dialysis tubes using a Hamilton syringe (Hamilton Company, Reno, Nevada). The ends of the hollow fibers were closed off by pinching with tweezers. The reactions enclosed by the dialysis fibers were then suspended in a beaker of sterile distilled water and allowed to equilibrate for 30 min. After 30 min the fibers were removed from the beaker, the pinched ends were cut and the contents of the hollow fiber

removed using the Hamilton syringe. Samples were then run on ABI 377s or a Megabace 1000 automated DNA sequencer.

The typical method of removing by-products of the sequencing reaction referred to as "cleaning up" is ethanol precipitation (EtOH prec.). The method of the present invention was compared to EtOH prec. As is evident from Figure 4, visual inspection of a 377 gel comparing results obtained with the dialysis procedures of the invention versus a standard EtOH prec. protocol that the dialysis methodology removes the unincorporated dye terminators which are by-products of sequencing reactions and produces better resolution compared to EtOH precip. A summary of the data is presented in Table 1. Column one describes the readlength which is the total number of reliable bases produced for that particular sequencing reaction. Columns two and three list the total number of bases which have quality scores that are greater than or equal to the Phred scores 30 and 20, respectively. The increase number of bases for the readlengths for dialysis coupled with the increased number of bases have a higher quality Phred scores indicates that the dialysis method produces superior results.

TABLE 1

	Readlength	Phred >30	Phred >20
Dialysis	797	438	602
EtOH prec	637	314	463

EXAMPLE III: Dialysis Kinetics

The following experiment demonstrates the kinetics of the dialysis reaction. Ten microliters of solution containing either BD terminator mix or ET primer mix was used as sample. The BD terminator mix simulates the excess fluorescently labeled dideoxy-terminator products found in a standard sequencing reaction, whereas the ET primer mix simulates the unincorporated primers found in a PCR reaction. Each sample was dialyzed using the above methods with a fibers having an average pore size of 100Kdal for the following time points: 0, 5,10, 15, 30, 60, 120 minutes and one final point at 12 hours or overnight (o/n). The results, as shown in Figure 5, clearly indicate that for the sequencing reactions (*i.e.*, BD terminator mix) dialysis occurs very quickly: equilibrium

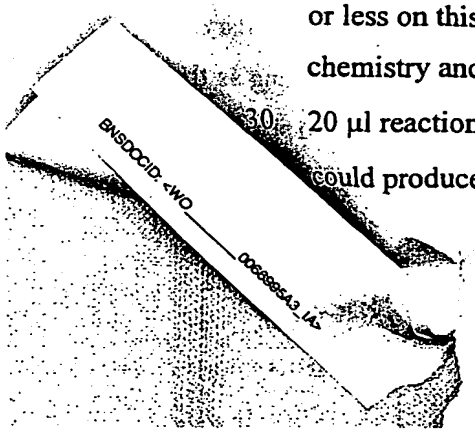
occurs within 5 to 10 min. Primers (*i.e.*, ET primer mix) which are much larger, take quite a bit longer to be removed, and in this experiment it took at least 60 min before equilibrium was reached. It should be noted that for the application of primer removal a hollow fiber with a larger pore size, *e.g.*, 500Kdal, is advantageously used.

EXAMPLE IV: Plasmid Purification

Plasmid template purification was performed using 1.1 mm ID diameter tubing having a pore size of 100Kdal. Plasmid subclones containing human BAC inserts were grown in 96 well format and processed by centrifugation, heating at >95°C for 2 min in STET buffer with lysozyme (75uL), and filtration. The cleared filtrate (~30 μL) was then dialyzed for 30 minutes against 1 mM EDTA. Four microliters of the resulting samples were then added directly to Big Dye terminator sequencing mix and the samples were separated on an ABI 377 automated DNA sequencer (PE Applied Biosystems, Foster City, CA). The results from 286 samples processed in this way gave an average clipped readlength of 703 nt and Phred-20 scores of 571 on passed sequences (with an overall pass rate of 85%). These results were essentially indistinguishable from those obtained using a standard automated plasmid preparation with quantification and reconfiguration in accordance with a modified Plasmid Minipreparation Protocol (Millipore Corp., Bedford, MA).

EXAMPLE V: Capillary-based Clean-up and Sample Handling

RapidCycler 1605 (Idaho Technology; Idaho Falls, ID) was tested for small volume thermocycling using glass, silica, TEFLON®, and stainless steel capillaries. Because heat transfer occurs through a low heat capacity medium (air) which can change temperature very quickly, PCR amplification can be accomplished in 30 minutes or less on this system. Sequencing experiments using with Big Dye Terminator chemistry and pGEM-3Zf(+) showed that reagent quantities as low as 1/40 of a standard 20 μl reaction (carried out in 1 μl volumes in glass, silica, or TEFLON® capillaries) could produce sequences of respectable quality and read length. Reactions carried out at



1/20 scale (2 μl) were quite robust and produced high quality reads (Phred-30 scores are shown in the accompanying table 2).

TABLE 2

Thermocyler Condition	Phred-30 score (unpurified sample)	Phred-30 score (purified sample)[c]
[a]Standard Thermocyling Reaction	358	425
[b]Fused Silica Capillary-silanized	285	478
[b]Fused Silica Capillary	433	422

[a] MJ Research Thermocycler, 2 μl reaction volume

[b] Fused Silica Capillaries: I.D.= 250μm (DB-1, J & W Scientific), 2 μl reaction vol.

[c] samples were purified on centri-sep columns

EXAMPLE VI: Capillary Sealing

Visual examination (using a microscope) of 100 nanoliter samples being heated inside a capillary revealed rapid sample dispersal unless both ends of the capillary were sealed (presumably due to rapid outgassing or localized boiling). In experiments with larger sample volumes where only one end of the tubing was effectively sealed by a syringe, the sample slug was observed to rapidly migrate back and forth in the tubing during thermocycling. This was assumed to be a result of the changing vapor pressure in the closed air space within the capillary tubing. The application of slight pressure to the open end of the tubing eliminated the movement and allowed successful thermocycling to be achieved in long TEFLON® capillary tubes. Therefore, sealing means, valves, or a pressure control system are a desirable part of a robust flow-through capillary thermocycling system.

EXAMPLE VII: Thermocycling Several Samples in a Single Capillary

Several samples were thermocycled at the same time in a single capillary. Samples were separated by means of air gaps within the capillaries. As long as the ends of the capillaries were sealed, the samples did not disperse and remained separate from each other. In one experiment, a capillary was loaded with four one-microliter samples,

each with a different dye primer reagent mix. These were thermocycled, pooled, and run on an ABI 377 automated DNA Sequencer (PE Applied Biosystems, Foster City, CA). The results showed that the four primer reactions were successful, and that they did not mix together during thermocycling.

EXAMPLE VIII: Mixing of Reagents

In one embodiment of the invention, sequencing reagents are advantageously added to the DNA prior to thermocycling in accordance with the reaction set up protocol. Traditionally, the dye is metered into a Microtiter plate, then the DNA is metered into the same plate. The plate is then mixed and thermocycled. In accordance with the invention, microliter quantities are thermocycled in a capillary. However it is difficult to aspirate this amount from a microtiter plate, such that it is desirable to include a Dye mix and DNA metering and mixing capability within the capillary. Tests were completed to show that metering and mixing of DNA and sequencing reagent can take place where the reagent is first taken up in a small capillary, and the end touched off, or rinsed, to reduce the amount of extra mix held up on the outside of the capillary. Next, the capillary was immersed into the DNA, and some DNA aspirated into the capillary. This caused very little loss of mix into the DNA source plate. As the DNA and reagent were further aspirated up the capillary, full mixing took place, allowing the sample to be successfully thermocycled. An alternative process was tested to pick up separate slugs of DNA and reagent mix separated by a small air gap and to push these out to the end of the pipette tip without touch-off. With an appropriate tip design (0.5 mm ID TEFLON® capillary tubing, for example) and liquid volume (2 microliters) the microdroplets adhered to the tip and mixed well in the process of coalescence and re-aspiration.

EXAMPLE IX: Sensitivity Testing

A test of overall sensitivity as well as the ability to recover small amounts of DNA from the dialysis tubing was performed as follows. A standard 1/4x Big Dye terminator reaction was carried out, and diluted to the equivalent of 1/32x, 1/64x, and

1/128x. Eleven replicates of the diluted samples were then dialyzed as described in Example II and separated on a MegaBace 100 automated DNA sequencer (Molecular Dynamics Corp., Sunnyvale, CA). The results indicated that the small amounts of sequencing reaction products present in these samples were effectively recovered in approximately 90% of the samples. The readlength and quality statistics are shown in the Table 3 (average of all samples).

TABLE 3

Dilution	Readlength	Phred-20	Phred-30
1/32x	601	526	460
1/64x	634	463	391
1/128x	562	463	383

References

Maxam AM, *et al.* (1977) A new method for sequencing DNA. Proc Natl Acad Sci U S A. 74: 560-4.

Bashkin, J., Roach, D., Leong, J., Bartosiewicz, M., Barker, D., Johnston, R.G. J. (1996) Capillary Electrophor. 3: 61-68.

Boddy, AV; Idle, JR. (1993) Cancer Surv., 17 (Pharmacokinetics and Cancer Chemotherapy), 79-104.

Boffa L.C., Carpaneto E.M. & Allfrey V.G., (1995).*Proc. Natl. Acad. Sci. USA* **92**, 1901

Caporaso, N.; Landi, M.T. (1995) Med. Lav., 86, 199-206.

Carrilho, E; Ruiz-Martinez, M., Berka, J., Smirnov, L., Goetzinger, W., Miller, A.w., Brady, D., Karger, B.L. (1996) Anal. Chem. 68: 3305-3313.

Dubiley S., Kirillov E., Lysov Y. & Mirzabekov A., (1997).*Nucleic Acid Res.* **25**, 2259

Carrilho, E., Miller, A., Ruiz Martinez, M.C., Kotler, L., Keisilman, j., Karger, B.L. (1997) B. L. Proc. SPIE 2985A, 4-18.

Egholm M., Buchard O., Christensen L., Behrens C., Freier S.M., Driver D.A., Berg R., Kim S.K., Norden B., & Nielsen P.E., (1993). *Nature* **365**, 566

Evensen, H. T.; Meldrum, D. R.; Cunningham, D. L. (1998) Rev. Sci. Instrum, 519-526.

Figeys D., Ahmadzedeh H., Arriaga E. & Dovichi N.J., (1996) *J. Chromatogr. A* **698**, 375.

Gingeras, TA.; Mack, D; Chee, MS.; Berno, AJ.; Stryer, L; Ghandour, G; Wang, C. PCT

Int. Appl., 132 pp. WO 9729212 A1 970814.

Haff L., Atwood J.G., . DiCesareJ, Katz E., Picozza E., Williams J.F. & Woudenberg T., *BioTechniques* **10**, 102 (1991).

Meldrum D., *et al.*, (1998) http://isdl.ee.washington.edu/GNL/acapella/

Hacia, JG.; Makalowski, W; Edgemon, K; Erdos, M R.; Robbins, CM.; Fodor, SPA.; Brody, LC.; Collins, FS. (1998) Nat. Genet., 18, 155-158.

Hacia, JG.; Brody, LC.; Chee, MS.; Fodor, SPA.; Collins, FS. (1996) Nat. Genet., 14, 441-447.

Hunicke-Smith, S.P. PCT Int. Appl., 42 pp. Application: WO 97-US10365 970616.

Hunicke-Smith, S.P. (1997) Ph.D. Dissertation, Stanford Univ., Stanford, CA, USA.

Witter C.T. & Garling D.J., *BioTechniques* **10**, 76 (1991).

Linder, M W.; Prough, R A.; Valdes, R, Jr. (1997) Clin. Chem., 43, 254-266.

Masson, E; Zamboni, WC. Can. (1997) Clin. Pharmacokinet., 32, 324-343.

Nickerson, D.A., Tobe, V.O., Taylor S.L. (1997) Nucleic Acids Res. 25: 2745-2751.

Nielsen P.E., Egholm M., Berg R.H., Buchard O., (1991).*Science* **254**, 1497

Parinov S., Barsky V., Yershov G., Kirillov E., Timofeev E., Belgovskiy A. & Mirzabekov A., (1996).*Nucleic Acid Res.* 24, 2998

PerSeptive Biosystem manual.

Rose D.J., *Anal. Chem* **65**, 3545 (1993).

Ruiz-Martinez, M.C., Salas-Solano O., Carrilho, E., Kotler, L., Karger, B.L. (1998) Anal. Chem. 70.

Salas-Solano O., Ruiz-Martinez, M.C., Carrilho, E., Kotler, L., Karger, B.L. (1998) Anal. Chem. 70.

Seeger C., Batz H.G. & Orum H., (1997). *BioTechniques* **23**, 512

Smith, D.R., Doucette-Stamm, L.A., Deloughery, C., *et al.*, (1997) J.Bact.179, 7135-55.

Stanford Technology Lab, (1998) http://sequence-www.stanford.edu/group/techdev/therm.htm

Swerdlow, H., Jones, B., Witter, C.T. (1997) Anal. Chem. 69: 848-855

Tan, H., Yeung, E.S. (1997) Analytical Chem. 69: 664-674.

Tan S.S. & Weis J.H., (1992) *PCR Methods and Applications*: CSH Laboratory Press, 137

Wang J., *J.Amer.Chem.Soc.* **118**, 7667 (1996).

Wang, K. Gan, L., Boysen C. & Hood, L. (1995).Anal. Biochem. 226, 85

Wang R., Cao W., Cerniglia C.E. & Jonson M.G., *The rapid Cyclist*, 12 (1995)

Weiler J., Gausepohl H., Hauser N., Jensen O.N., & Hoheisel J.D., (1997).*Nucleic Acid Res.* **25**, 2792

Incorporation By Reference

All patents, published patent applications and other references disclosed herein are hereby expressly incorporated herein in their entireties by reference.

Equivalents

Those skilled in the art will recognize, or will be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

What is claimed is:

1. A device for integrated processing of a biological sample, said device comprising

a first capillary tube sized for holding the biological sample, said first capillary tube having a first end and a second end,

a second capillary tube sized for holding said biological sample, said second capillary tube having a first end and a second end, and

a connector coupled to the second end of the first capillary tube and the first end of the second capillary tube for mechanically and fluidly connecting the first and second capillary tubes together.

2. The device of claim 1, wherein said connector is a fluid regulating element for selectively providing a fluid seal between the first and second capillary tubes.

3. The device of claim 2, wherein said fluid regulating element comprises a valve or a pinchable silicone connector.

4. The device of claim 1, wherein said second capillary tube is adapted to be removably and replaceably coupled to the first capillary tube by the connector.

5. The device of claim 1, wherein said second capillary tube is adapted to be removably and replaceably coupled to said connector, said connector being adapted to selectively place said first and second capillary tubes in fluid communication with each other.

6. The device of claim 1, further comprising a support assembly for supporting the first capillary tube at said first end.

7. The device of claim 6, wherein said support assembly comprises
a fluid handling element fluidly coupled to said first end of said first capillary tube, and
a pump for forcing fluid into and out of the fluid handling element.

8. The device of claim 7, further comprising a second connector coupled to the first end of the first capillary tube and disposed between the fluid handling element and the capillary tube.

9. The device of claim 7, wherein said fluid handling element comprises a syringe-type fluid housing.

10. The device of claim 7, wherein said fluid handling element comprises at least a portion of a syringe.

11. The device of claim 8, wherein said second connector comprises a fluid regulating element.

12. The device of claim 1, further comprising a fluid tip coupled to said second end of said second capillary device for introducing fluid to or dispensing fluid from said second capillary tube.

13. The device of claim 1, further comprising
a second connector coupled to the second end of the second capillary tube, and
a fluid tip coupled to said second connector for forming an entrance or exit aperture in said device for introducing fluid to or dispensing fluid from said second capillary tube, wherein said second connector selectively forms a fluid seal between the fluid tip and the second capillary tube.

14. The device of claim 1, wherein said first capillary tube comprises a glass capillary tube.

15. The device of claim 1, wherein said first capillary tube comprises a silica capillary tube.

16. The device of claim 1, wherein said first capillary tube is coated with a polyimide material.

17. The device of claim 1, wherein said first capillary tube is coated with a polyimide material to facilitate temperature processing of the biological sample when resident within the first capillary tube.

18. The device of claim 1, wherein said connector comprises a valve disposable between an open position for placing said first capillary and said second capillary tube in fluid communication with each other, and a closed position for fluidly sealing said first capillary tube from said second capillary tube.

19. The device of claim 1, wherein said second capillary tube comprises one or more membrane elements for separating components in the biological sample when resident within the second capillary tube.

20. The device of claim 19, wherein said membrane element comprises a semi-permeable microfiber.

21. The device of claim 1, wherein said first and second capillary tubes are sized and configured for holding said biological sample occupying a volume ranging from 10 μl to 0.05 μl.

22. A device for integrated processing of a biological sample, said device comprising

a first capillary tube sized for holding the biological sample, said first capillary tube having a first end and a second end,

a first regulating element coupled to the first end of the first capillary tube for selectively providing a fluid seal thereat,

a second capillary tube sized for holding said biological sample, said second capillary tube having a first end and a second end, and

a second fluid regulating element disposed between the second end of the first capillary tube and the first end of the second capillary tube for selectively providing a fluid seal between the first and second capillary tubes.

23. The device of claim 22, wherein said second capillary tube is adapted to be removably and replaceably coupled to the second fluid regulating element.

24. The device of claim 22, wherein said second capillary tube is adapted to be removably and replaceably coupled to the second fluid regulating element, and said second fluid regulating element is adapted to selectively place said first and second capillary tubes in fluid communication with one another.

25. The device of claim 22, further comprising a support assembly for supporting the first capillary tube at said first end.

26. The device of claim 25, wherein said support assembly comprises

a fluid handling element fluidly coupled to said first end of said first capillary tube, and

a pump for forcing fluid into and out of the fluid handling element.

27. The device of claim 26, wherein said fluid handling element comprises a syringe-type fluid housing.

28. The device of claim 26, wherein said fluid handling element comprises at least a portion of a syringe.

29. The device of claim 22, further comprising a fluid tip coupled to said second end of said second capillary device for introducing fluid to or dispensing fluid from said second capillary tube.

30. The device of claim 22, further comprising

a third fluid regulating element coupled to the second end of the second capillary tube, and

a fluid tip fluidly coupled to said third fluid regulating element and forming an entrance or exit aperture in said device for introducing fluid to or dispensing fluid from said second capillary tube, wherein said third fluid regulating element selectively forms a fluid seal between the fluid tip and the second capillary tube.

31. The device of claim 22, wherein said first capillary tube comprises one of a glass and a silica capillary tube.

32. The device of claim 22, wherein said first capillary tube is coated with a polyimide material.

33. The device of claim 22, wherein said first capillary tube is coated with a polyimide material to facilitate thermal processing of the biological sample when resident within the first capillary tube.

34. The device of claim 22, wherein said first fluid regulating element comprises a valve disposable between an open position for placing said first capillary and said second capillary tube in fluid communication with each other, and a closed position for fluidly sealing said first capillary tube from said second capillary tube.

35. The device of claim 22, wherein said first or second fluid regulating elements comprises a pinchable silicone connector.

36. The device of claim 22, wherein said second capillary tube comprises one or more membrane elements for separating components in the biological sample when resident within the second capillary tube.

37. The device of claim 36, wherein said membrane element comprises a semi-permeable microfiber.

38. The device of claim 22, wherein said first and second capillary tubes are sized and configured for holding microliters of the selected fluid.

39. A device for integrated processing of a biological sample, said device comprising

a first capillary tube sized for holding the biological sample and adapted for thermal processing the sample, said first capillary tube having a first end and a second end,

a second capillary tube sized for holding said biological sample, said second capillary tube having a first end and a second end, and

a fluid regulating element coupled to the second end of the first capillary tube and the first end of the second capillary tube for mechanically and fluidly connecting the first and second capillary tubes together.

40. A modular device for integrated processing of a biological sample, said device comprising

a first modular array of capillary tubes for holding the biological sample, said first modular array having a first array end and a second array end,

a second modular array of capillary tubes for holding the biological sample, said second modular array coupled to said second array end of said first modular array, wherein said second modular array is removably and replaceably coupled to said first modular array, and

an array of fluid regulating elements coupled to the second array end of the first modular array and a first end of said second modular array for selectively providing a fluid seal between the first and second modular arrays of capillary tubes.

41. The modular device of claim 40, further comprising a support assembly coupled to the first array end of the first modular array for supporting the array at said first end,

42. A system for automatic and integrated processing of a biological sample, said system comprising

a first capillary tube for holding the biological sample, said first capillary tube having a first end and a second end,

a support assembly for supporting the capillary tube at said first end,

a second capillary tube for holding said biological sample, said second capillary tube having a first end and a second end,

a first fluid regulating element disposed between the second end of the first capillary tube and the first end of the second capillary tube for selectively providing a fluid seal between the first and second capillary tubes, and

a plate handling assembly positioned for selectively disposing a fluid reservoir beneath the second capillary tube during operation.

43. The system of claim 42, further comprising a second fluid regulating element disposed between the support element and the first end of the first capillary tube for selectively providing a fluid seal between the support element and the first capillary tube,

44. A system for automatic and integrated processing of a biological sample, said system comprising

a first capillary tube for holding the biological sample, said first capillary tube having a first end and a second end,

a support assembly for supporting the capillary tube at said first end,

a first fluid regulating element disposed between the support element and the first end of the first capillary tube for selectively providing a fluid seal between the support element and the first capillary tube,

a second capillary tube for holding said biological sample, said second capillary tube having a first end and a second end,

a second fluid regulating element disposed between the second end of the first capillary tube and the first end of the second capillary tube for selectively providing a fluid seal between the first and second capillary tubes, and

a plate handling assembly positioned for selectively disposing a fluid reservoir beneath the second capillary tube during operation.

45. The system of claim 44, wherein said plate handling assembly comprises a support surface for supporting a fluid reservoir, and means for moving the fluid reservoir into the selected position.

46. The system of claim 44, further comprising a controller in communication with one of said fluid regulating elements for automatically positioning said regulating element between an open position for placing said first capillary and said second capillary tube in fluid communication with each other, and a closed position for fluidly sealing said first capillary tube from said second capillary tube.

47. The system of claim 45, wherein said second capillary tube is adapted to be removably and replaceably coupled to the second fluid regulating element, and said second fluid regulating element is adapted to selectively place said first and second capillary tubes in fluid communication with each other.

48. The system of claim 44, further comprising

a third fluid regulating element coupled to the second end of the second capillary tube, and

a fluid tip coupled to said third fluid regulating element and forming an entrance or exit aperture in said device for introducing fluid to or dispensing fluid from said second capillary tube, wherein said third fluid regulating element selectively forms a fluid seal between the fluid tip and the second capillary tube.

49. The system of claim 44, wherein said support assembly comprises a fluid handling element fluidly coupled to said first end of said first capillary tube, and a pump for forcing fluid into and out of the fluid handling element.

50. The system of claim 44, wherein said first capillary tube comprises one of a glass and a silica capillary tube.

51. The system of claim 44, wherein said first capillary tube is coated with a polyimide material.

52. The system of claim 44, wherein said first capillary tube is coated with a polyimide material to facilitate temperature processing of the biological sample when resident within the first capillary tube.

53. The system of claim 44, wherein said first fluid regulating element comprises a valve disposable between an open position for placing said first capillary and said second capillary tube in fluid communication with each other, and a closed position for fluidly sealing said first capillary tube from said second capillary tube.

54. The system of claim 44, wherein said first or second fluid regulating elements comprise a pinchable silicone connector.

55. The system of claim 44, wherein said second capillary tube comprises one or more membrane elements for separating components in the biological sample when resident within the second capillary tube.

56. The system of claim 55, wherein said membrane element comprises a semi-permeable microfiber.

57. The system of claim 44, wherein said first and second capillary tubes are sized and configured for holding microliters of the selected fluid.

58. A modular system for automatically processing a biological sample, said system comprising

a first modular array of capillary tubes for holding the biological sample, said first modular array having a first array end and a second array end,

a support assembly coupled to the first array end of the first modular array for supporting the array at said first end,

a second modular array of capillary tubes for holding the biological sample, said second modular array coupled to said second array end of said first modular array, wherein said second modular array is removably and replaceably coupled to said first modular array, and

a plate handling assembly positioned for selectively disposing a fluid reservoir beneath the second modular array of capillary tubes during operation.

59. The modular system of claim 58, further comprising a first array of fluid regulating elements coupled to the second array end of the first modular array and the first end of said second modular array for selectively providing a fluid seal between the first and second modular arrays of capillary tubes.

60. The modular system of 59, further comprising a second array of fluid regulating elements coupled to the first array end of the first modular array and the support assembly for selectively providing a fluid seal between the support element and the first capillary tube.

61. The device of claim 1, wherein said first capillary tube is adapted for temperature processing of said sample.

62. The device of claim 1, wherein said first capillary tube is adapted for thermocycling of said sample.

63. The device of claim 1, wherein said second capillary tube comprises separation means for purifying said sample by molecular size discrimination.

64. The device of claim 63, wherein said separation means comprises a semi-permeable microfiber for dialysis of said sample.

65. The device of claim 1 further comprising means for passing a fluid over said first capillary tube.

66. The device of claim 1, wherein said first capillary tube is adapted for thermocycling of said sample, and said second capillary tube comprises separation means for dialysis of said sample.

67. The device of claim 66, wherein said biological sample comprises a polynucleotide, polypeptide, carbohydrate, or mixtures thereof.

68. The device of claim 67, wherein said polynucleotide comprises DNA.

69. A device for dialysis of a biological microsample, said device comprising a capillary tube sized for holding said microsample and comprising separation means for purifying said microsample by molecular size discrimination.

70. A modular device for dialysis of a biological microsample, said device comprising a modular array of capillary tubes sized for holding said microsample, each of said tubes comprising separation means for purifying said microsample by molecular size discrimination.

71. A device for integrated processing of a biological microsample, said device comprising

a first capillary tube sized for holding the biological microsample and adapted for thermocycling the sample, said first capillary tube having a first end and a second end,

a second capillary tube sized for holding said biological sample and adapted for dialysis of the sample, said second capillary tube having a first end and a second end, and

a connector coupled to the second end of the first capillary tube and the first end of the second capillary tube for mechanically and fluidly connecting the first and second capillary tubes together.

72. The device of claim 71, wherein said second capillary tube comprises separation means for purifying the sample by molecular size discrimination.

73. The device of claim 72, wherein said separation means comprises a semi-permeable microfiber.

74. A modular device for integrated processing of a biological microsample, said device comprising

a first modular array of capillary tubes sized for holding the biological microsample and adapted for thermocycling said sample, said first modular array having a first array end and a second array end,

a second modular array of capillary tubes sized for holding the biological microsample and adapted for dialysis of said sample, said second modular array having a first array end and a second array end, wherein said second modular array is coupled to said second array end of said first modular array, and wherein said second modular array is removably and replaceably coupled to said first modular array, and

an array of connectors coupled to the second array end of the first modular array and the first end of said second modular array for selectively providing a fluid seal between the first and second modular arrays of capillary tubes.

75. A method for processing a biological sample, said method comprising the steps of

providing a first capillary tube sized for holding the biological sample,

providing a second capillary tube sized for holding said biological sample,

mechanically and fluidly coupling a second end of the first capillary tube and a first end of the second capillary tube together,

supporting the first capillary tube at a first end, and

positioning a fluid reservoir beneath the second capillary tube during operation.

76. A method for integrated processing of a biological microsample comprising

temperature treating said microsample, and

purifying said microsample,

wherein said temperature treating and purifying steps are carried out in a device that integrates said steps, such that integrated processing of said microsample is achieved.

77. The method of claim 76, wherein said device is a device according to claim 71.

78. The method of claim 76 wherein said device is a device according to claim 74.

79. The method of claim 76, wherein said temperature treating step comprises thermocycling and said purifying step comprises dialysis.

80. The method of claim 79, wherein said microsample comprises a polynucleotide, polypeptide, carbohydrate, or mixtures thereof.

81. The method of claim 80, wherein said polynucleotide comprises DNA.

82. The method of claim 81, wherein said microsample is dialyzed to removed unwanted components of a reaction selected from the group consisting of polymerase chain reactions, DNA sequencing reactions, oligonucleotide extension reactions, exonuclease reactions, OLA reactions, hybridization reactions, and allele-specific polymerase chain reactions.

83. The method of claim 80, wherein said microsample occupies a volume ranging from 10 μl to 0.05 μl

84. A method for conducting dialysis on a biological microsample comprising introducing said microsample into a capillary tube which comprises separation means for purifying the sample by molecular size discrimination, and allowing said microsample to reside in said capillary tube for a time sufficient such that dialysis of said sample is achieved.

85. The method of claim 84, wherein said dialysis is conducted to remove undesired components of a reaction selected from the group consisting of polymerase chain reactions, DNA sequencing reactions, oligonucleotide extension reactions, exonuclease reactions, OLA reactions, hybridization reactions, and allele-specific polymerase chain reactions.

86. The method of claim 84, wherein said microsample comprises a polynucleotide, polypeptide, carbohydrate, or mixtures thereof.

87. The method of claim 86, wherein said polynucleotide comprises DNA.

88. The method of claim 84, wherein said microsample occupies a volume ranging from 10 μl to 0.05 μl.

89. The method of claim 84, wherein said separation means comprises one or more membrane elements.

90. The method of claim 89, wherein said membrane element comprises a semi-permeable microfiber.

91. The method of claim 90, wherein said microfiber is a hollow fiber dialysis tube.

92. The method of claim 91, wherein said dialysis tube has a molecular weight cut-off about 100 Kdal.

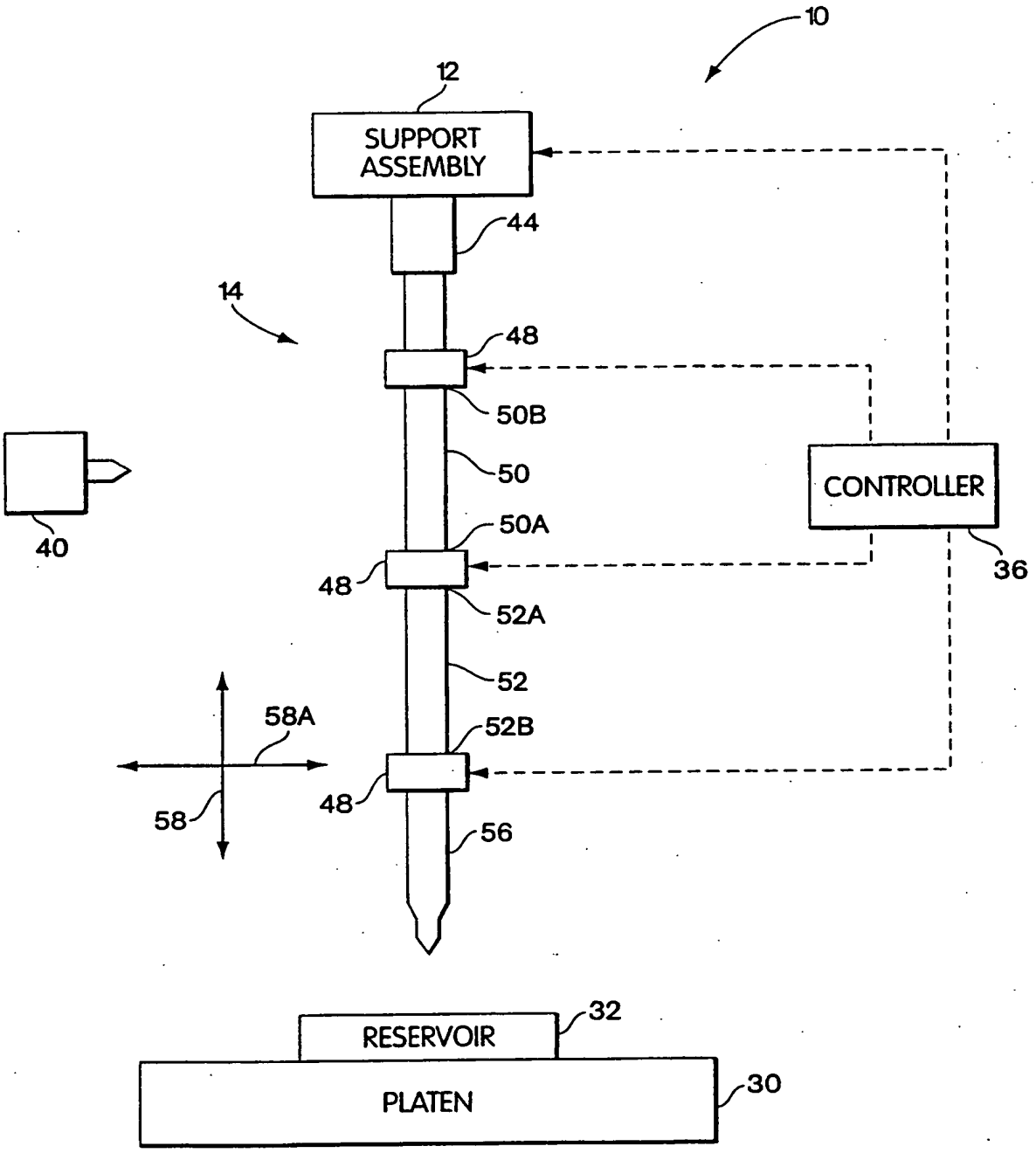


Fig. 1

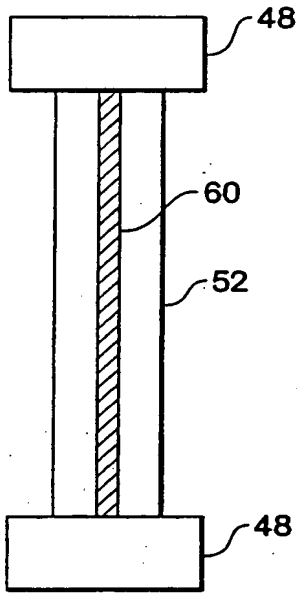


Fig. 2

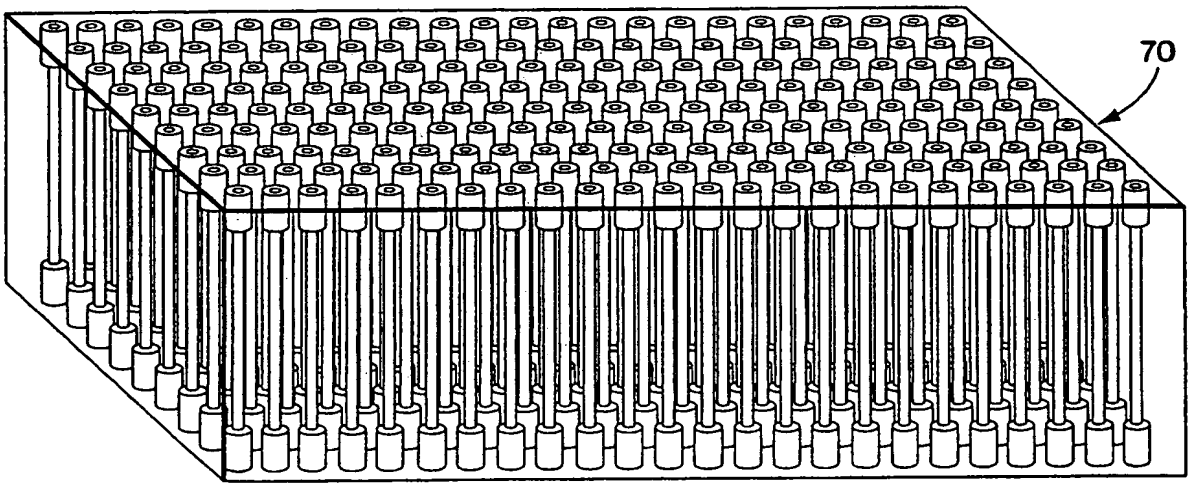


Fig. 3

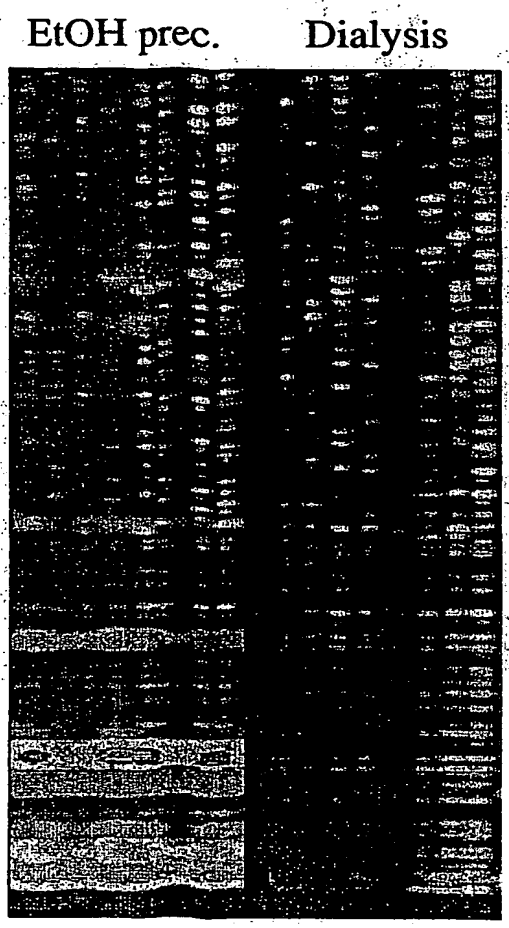


Fig. 4

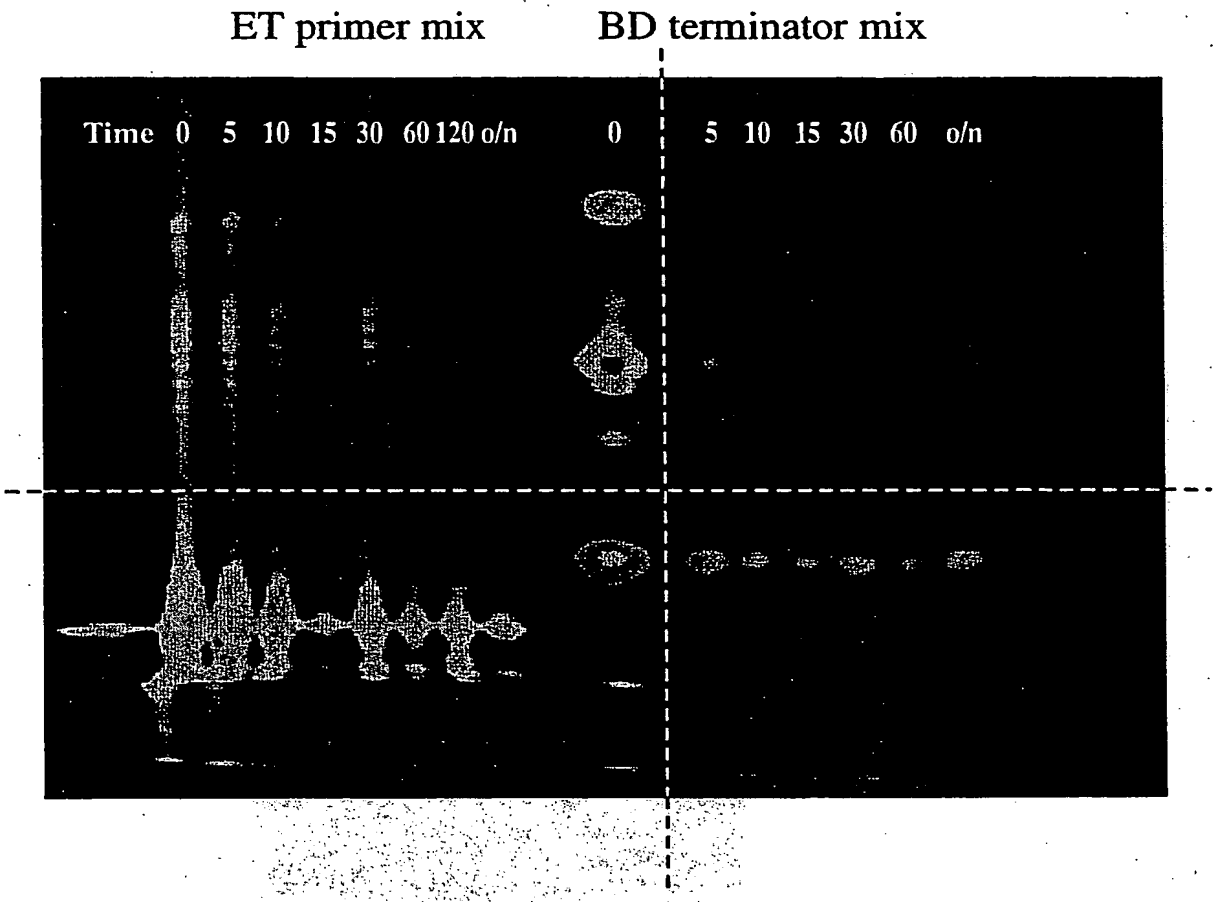


Fig. 5

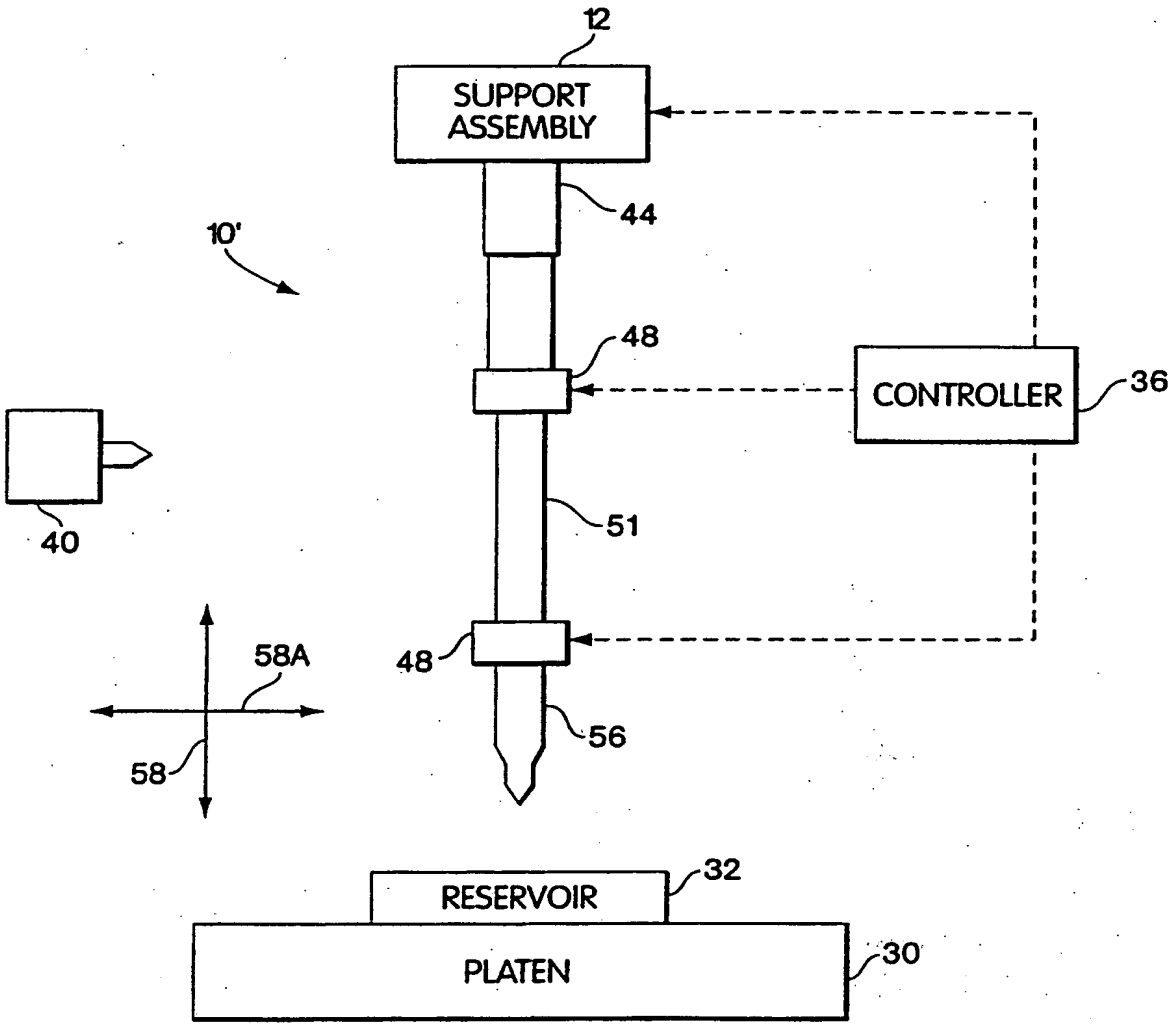

12
SUPPORT ASSEMBLY
44
10'
48
CONTROLLER
36
40
51
58A
48
56
58
RESERVOIR
32
PLATEN
30

Fig. 6

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/11371

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01L3/00 C12Q1/68 B01J19/00 B01L7/00 B01L3/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B01L C12Q G01N B01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, IBM-TDB

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 769 216 A (CHANDLER HOWARD M ET AL) 6 September 1988 (1988-09-06) column 2, line 5 - line 21 column 7, line 16 -column 8, line 27; figure 2 ---	1,6-10, 12,14, 15,21,75
X	US 5 449 064 A (HOGAN BARRY L ET AL) 12 September 1995 (1995-09-12) column 6, line 65 -column 7, line 63 ---	1-3
X	WO 99 13312 A (MOLECULAR DYNAMICS INC) 18 March 1999 (1999-03-18) page 1, line 29 -page 2, line 3 page 2, line 32 - line 36 page 3, line 9 - line 20 figures 11-13; examples 1-3 figure 15; example 4 --- -/--	1-3

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 October 2000	12.01.01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	HOCQUET, A

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/11371

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97 48818 A (UNIV LELAND STANFORD JUNIOR) 24 December 1997 (1997-12-24) page 7, line 25 -page 8, line 12 page 9, line 15 -page 10, line 11 page 11, line 9 - line 14 page 13, line 31 - line 33	1,40
A	EP 0 903 176 A (SQUIBB BRISTOL MYERS CO) 24 March 1999 (1999-03-24) paragraph [0017]; figures paragraph [0077] paragraph [0080] - paragraph [0081]	2,40
A	US 5 856 174 A (FODOR STEPHEN P A ET AL) 5 January 1999 (1999-01-05) column 29, line 64 -column 30, line 14	2
A	US 5 030 555 A (CLEMMONS ROGER M) 9 July 1991 (1991-07-09) column 5, line 5 - line 61; figures	1
A	EP 0 805 350 A (UNI DEGLI STUDI DI MILANO) 5 November 1997 (1997-11-05) page 3, line 43 - line 55 page 4, line 39 -page 5, line 18; claim 6 page 5, line 38 - line 43	1,19,55, 56,74
A	EP 0 616 218 A (HITACHI LTD) 21 September 1994 (1994-09-21) column 4, line 16 - line 47; figure 1 column 10, line 16 - line 35; figure 8	1,22,39, 40,42, 44,58, 74,75

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

In' ational application No.
PCT/US 00/11371

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-21,22-38,39,40-41,42-43,44-57,58-60,74,75

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-21,22-38,39,40-41,42-43,44-57,58-60,74,75

   devices and methods for processing a sample comprising capillary tubes connected by a fluid regulating element for selectively providing a fluid seal between the capillary tubes

2. Claims: 63, 64,69-73,84-92

   device ans methods for dialysis

3. Claims: 61,62,65-68,76-83

   method for processing a microsample comprising temperature treating and purifying said microsample

INTERNATIONAL SEARCH REPORT

ormation on patent family members

International Application No
PCT/US 00/11371

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4769216 A	06-09-1988	AT 27179 T	15-05-1987
		AT 41528 T	15-04-1989
		AU 553422 B	17-07-1986
		AU 7867881 A	01-07-1982
		WO 8202211 A	08-07-1982
		CA 1184847 A	02-04-1985
		CA 1191786 A	13-08-1985
		DE 3176186 D	19-06-1987
		DE 3177011 D	20-04-1989
		DK 2590 A,B	05-01-1990
		DK 375782 A,B,	20-08-1982
		EP 0067182 A	22-12-1982
		EP 0134605 A	20-03-1985
		JP 57502041 T	18-11-1982
		NO 822750 A	12-08-1982
		NO 904856 A	12-08-1982
		NZ 199286 A	09-05-1986
		NZ 212930 A	20-02-1987
		US 4590157 A	20-05-1986
US 5449064 A	12-09-1995	NONE	
WO 9913312 A	18-03-1999	EP 1019694 A	19-07-2000
WO 9748818 A	24-12-1997	EP 0927265 A	07-07-1999
		US 6132996 A	17-10-2000
		US 5985651 A	16-11-1999
EP 0903176 A	24-03-1999	US 5961925 A	05-10-1999
		CA 2246088 A	22-03-1999
US 5856174 A	05-01-1999	AU 6404996 A	05-02-1997
		EP 0843734 A	27-05-1998
		JP 11509094 T	17-08-1999
		WO 9702357 A	23-01-1997
		US 6043080 A	28-03-2000
		US 5922591 A	13-07-1999
US 5030555 A	09-07-1991	AU 4300089 A	02-04-1990
		WO 9002950 A	22-03-1990
EP 0805350 A	05-11-1997	GB 2312750 A	05-11-1997
		AT 194427 T	15-07-2000
		DE 69702423 D	10-08-2000
		DE 69702423 T	09-11-2000
		US 5976465 A	02-11-1999
EP 0616218 A	21-09-1994	JP 6265447 A	22-09-1994
		US 5480614 A	02-01-1996